El efecto de los depredadores en el comportamiento y en la respuesta fisiológica de estrés en el conejo (*Oryctolagus cuniculus*)

Tesis Doctoral

Raquel Monclús Burgoa

UNIVERSIDAD AUTÓNOMA DE MADRID

FACULTAD DE CIENCIAS

DEPARTAMENTO DE BIOLOGÍA

El efecto de los depredadores en el comportamiento y en la respuesta fisiológica de estrés en el conejo (*Oryctolagus cuniculus*)

Memoria presentada para optar al Grado de Doctor por

Raquel Monclús Burgoa

Bajo la dirección de los Doctores:

Francisco Javier de Miguel Águeda
Profesor Titular
Departamento de Biología
Universidad Autónoma de Madrid
España

Heiko G. Rödel
Profesor Asociado
Departamento de Fisiología Animal
Universidad de Bayreuth
Alemania

Madrid, 2007

The rabbit has a charming face;
Its private life is a disgrace.
I really dare not name to you,
The awful things that rabbits do.

Richard Adams, “Watership Down”

Agradecimientos

Durante un cuarto de mi vida he estado trabajando en la proto-tesis, pre-tesis y tesis. Es mucho tiempo y muchas las personas que han compartido esta etapa conmigo. Unos cuantos siempre estuvieron, algunos han ido llegando y otros se fueron. A todos ellos les debo que yo me encuentre en estos momentos escribiendo estos agradecimientos, por lo que son los absolutos protagonistas. Gracias.

En particular, me gustaría agradecer a los directores de esta tesis, Javier de Miguel y Heiko Rödel, la confianza que siempre depositaron en mí, la paciencia y todo lo que me han enseñado. Soy muy afortunada porque siempre han estado a mi lado y de mi lado, incondicionalmente. Sin lugar a dudas esto ha sido un trabajo de tres.

Tampoco habría sido posible esta tesis sin el apoyo del doctor von Holst, Profesor del Departamento de Fisiología de la Universidad de Bayreuth, donde he realizado los experimentos. Él no creía en la depredación, y aún así no dudó en ofrecerme todas las facilidades para realizar mis experimentos. Espero haberle convertido. También me gustaría expresar mi agradecimiento al doctor Palme, de la Universidad de Veterinaria de Viena, por confiar en el proyecto y poner a mi disposición su laboratorio, su equipo, y su conocimiento.

Muchas gracias a mis compañeros y amigos de la Unidad de Zoología (y alrededores) de la UAM, de la Universidad de Bayreuth y de la Universidad de Veterinaria de Viena. Han hecho mucho más divertido todo el proceso.

Me gustaría agradecer a quienes sin su apoyo incondicional esta tesis no habría sido posible: mis padres, porque me enseñaron a pelear y siempre están ahí para curarme las heridas. A mi hermano, al resto de la familia, y a los amigos, les agradezco que me hayan acompañado anímicamente en esta montaña rusa de picos, valles y algunas simas abisales.

Y por supuesto estoy muy agradecida a la Comunidad de Madrid por concederme una beca predoctoral, al programa de Estancias cortas de dicho organismo, a la Universidad Autónoma de Madrid y al DAAD, por su programa de ayudas para jóvenes investigadores, porque no sólo de conejos vive el hombre.

Índice

CAPÍTULO 1

Introducción. El reconocimiento de los depredadores y las respuestas antidepredatorias

La depredación constituye una fuerza de selección decisiva, y probablemente constituya uno de los procesos que pueden modificar más drásticamente el éxito biológico de una especie. Para la mayoría de los animales, fracasar durante una época reproductiva o no comer durante un día puede conllevar efectos negativos, pero errar en un encuentro con un depredador modifica de manera dramática su éxito biológico. En las especie presa han aparecido una serie de adaptaciones que incrementan la probabilidad de escapar con éxito de semejante situación. Las estrategias pueden ser morfológicas (Caro 2005), fisiológicas (Flier *et al*. 1980) o comportamentales (Kats y Dill 1998), y tienen que ver con alguna de las etapas de la secuencia antidepredatoria. La depredación consta de un conjunto de pautas que concluyen con el consumo de la presa. La secuencia depredatoria podría resumirse en las siguientes etapas: encuentro (entendiendo como encuentro la coincidencia espacio-temporal del depredador y de la presa), detección, identificación, acercamiento, subyugación y consumo de la presa (Endler 1991). Para una presa, la probabilidad de escapar con éxito disminuye según se suceden los diferentes estadíos de la secuencia depredatoria (Lima y Dill 1990). Es decir, la probabilidad para una presa de salir airosa del encuentro con un depredador suele ser mayor en las etapas de encuentro y detección que en la de subyugación. Por lo tanto, la mayoría de las presas poseen mecanismos antidepredatorios que actúan en las primeras etapas. Muchos de ellos consisten en la detección temprana del depredador. Si las presas pueden detectar al depredador sin que éste las detecte, las opciones de escapar con éxito se incrementarán notablemente, ya que así podrán evitar los encuentros directos, que suelen ser tan peligrosos. Para ello, se tienen que dar dos circunstancias: (1) tienen que disponer de mecanismos de reconocimiento de sus depredadores y (2) han ser capaces de evaluar el riesgo actual de depredación. Una vez que las presas han valorado que existe un riesgo (3) deben desarrollar una estrategia antidepredatoria adecuada.

A continuación se va a realizar una revisión general de los métodos que permiten a los animales reconocer a sus depredadores y evaluar el riesgo de depredación, así como de los tipos de respuestas que las especies presa exhiben para intentar reducir dicho riesgo. Aunque se han intentado abarcar todos los grupos, la revisión se ha centrado especialmente en los mamíferos, dado el número de trabajos que han originado. Por la misma razón, se ha hecho más hincapié en el reconocimiento de los depredadores

por el olor, dado que éste es el canal químico más utilizado en la mayoría de los mamíferos.

1. Reconocimiento de los depredadores

Para adquirir información espacial y temporal fiable acerca de lo que sucede en el entorno, los animales pueden recurrir a claves visuales, acústicas u olfativas. Sin embargo, la mayoría de las especies utilizan principalmente sólo un canal, que suele ser el que está más desarrollado según sus modos de vida. La adquisición de información es sumamente importante, y en condiciones ambientales cambiantes los animales variarán el modo de adquisición de información. Por ejemplo, algunos peces utilizan las señales químicas cuando la turbidez del agua aumenta, y por lo tanto la visibilidad disminuye, mientras que en condiciones de mejor visibilidad dependerán en mayor medida de las claves visuales (compensación sensorial: Hartman y Abrahams 2000). La inspección visual de los depredadores puede proporcionar información incluso sobre la motivación de los depredadores. Así, algunos peces pueden diferenciar depredadores hambrientos y saciados por medio de las posturas que adoptan (Licht 1989). De forma similar, las rapaces que están buscando una presa pueden reconocerse porque llevan los ojos más cerrados, las plumas de la cabeza más aplastadas y las alas algo más elevadas (Inglis y Shepherd 1990). Muchas aves son diurnas y adquieren información mayoritariamente por los canales visual (van der Veen 2002; Fernández-Juricic y Kacelnik 2004) y auditivo (Adams *et al*. 2006). En los decápodos terrestres y semiterrestres la mayoría de las interacciones inter e intraespecíficas dependen de señales visuales. En un experimento con cangrejos de los manglares (*Sesarma leptosoma*), Cannicci y colaboradores (2002) constataron que éstos reconocían a su principal depredador, el cangrejo *Epixanthus dentatus*, por la forma y por la presencia de las pinzas abiertas. De manera similar, se cree que el reconocimiento de la mayoría de los carnívoros se debe a que todos comparten una fisonomía similar (ojos en posición frontal, orejas puntiagudas). Se cree que estas características son reconocidas por las especie presa aunque no haya estado nunca en contacto con depredadores (Curio 1993; Topál y Csányi 1994; Blumstein *et al*. 2000).

El reconocimiento de los depredadores por claves químicas es un tema que ha originado un gran número de investigaciones, por su importancia aplicada en el control químico de plagas (Sullivan *et al*. 1988 a, b; Calder y Gorman 1991; Epple *et al*. 1993;

Boag y Mlotkiewicz 1994; Burwash *et al*. 1998 a, b). Además, dado que el emisor y el receptor no suelen coincidir en el mismo lugar en el momento del intercambio de información, el uso de estas claves constituiría una forma segura de obtener información del depredador, evitando los encuentros directos y por lo tanto maximizando la probabilidad de la presa de escapar con éxito (Lima y Dill 1990). Los resultados obtenidos en dichos trabajos se resumen, por grupos, a continuación:

Filo Artrópodos

Clase Arácnidos

Los ácaros reconocen a los depredadores por medio de la presencia en sus excreciones de ciertos metabolitos resultantes de la digestión de la carne (Grostal y Dicke 2000). Sin embargo, existen evidencias de que también reconocen compuestos procedentes de congéneres heridos (hemolinfa, feromonas de alarma) (Grostal y Dicke 1999).

Clase Branquiópodos

Boriss y colaboradores (1999) concluyeron que la trimetilamina (TMA) era la responsable del comportamiento antidepredatorio de *Daphnia hyalina*. Sin embargo, la TMA es una sustancia muy común en la naturaleza (King 1984). Se produce como resultado de muchos procesos de descomposición, e incluso por la actividad del zooplancton, por lo que probablemente sólo podría desencadenar una respuesta antidepredatoria en presencia de otras sustancias (no identificadas).

Filo Cordados

Clase Peces

En los peces, la inspección olorosa de los depredadores para adquirir información sobre el riesgo actual de depredación está muy extendida. Los peces pertenecientes al Superorden Ostariofisios y algunos Acantopterigios emiten al ser heridos unas secreciones de alarma (Schreckstoff) que desencadenan un comportamiento antidepredatorio en los congéneres (von Frisch 1941). En los peces ostariofisios, estas secreciones se encuentran en unas células caliciformes de la epidermis del animal, mientras que en los pércidos se encuentran en unas células análogas. En las secreciones de los ostariofisios se ha aislado una feromona de alarma, la hipoxantina 3-(N)-óxido (Pfeiffer *et al*. 1985). La liberación de estas sustancias se produce normalmente por el daño mecánico de la piel del animal, ya sea producido por un depredador o no. En los

peces de estos superórdenes, la presencia de estas señales de alarma en las excreciones del depredador también parece constituir una clave importante para desarrollar un comportamiento antidepredatorio (Brown *et al*. 2000; Brown y Zachar 2002). Las señales son reconocibles por diferentes especies siempre y cuando posean señales de alarma, y las especies compartan un hábitat y una presión de depredación similar (Mathis y Smith 1993).

Clase Anfibios

Las larvas de anfibios poseen señales de alarma similares a las de los peces (Hews 1988) que son capaces de reconocer en las excreciones del depredador. Por el contrario, en los anfibios adultos, predominantemente terrestres, muestran respuestas de miedo ante ciertos lípidos no volátiles secretados por el tejido epidérmico de las serpientes, sus principales depredadores (Flowers y Graves 1997). Los pletodóntidos poseen feromonas de alarma asociadas a células epidérmicas (Lutterschmidt *et al*. 1994), y su presencia en los excrementos de los depredadores provoca un comportamiento antidepredatorio. Por ejemplo, las salamandras rojas (*Plethodon cinereus*) desarrollan una respuesta mayor al olor de las serpientes alimentadas con salamandras que al de las serpientes alimentadas con lombrices (Murray y Jenkins 1999).

Clase Reptiles

Dial y Schwenk (1996) demostraron que los geckos *Coleonyx brevis* reaccionaban a compuestos químicos de la piel de diferentes serpientes depredadoras (*Gyalopium canum*, *Sonoro semiannulata*, *Phyllorhyncus decurtatus*, *Hypsiglena torquata*).

Clase Aves

No existen muchos trabajos sobre el reconocimiento de los depredadores por parte de las aves porque durante mucho tiempo se las ha considerado anósmicas o microsmáticas (Jones & Roper 1997). Sin embargo, la anatomía del sistema olfativo de las aves es muy similar a la del resto de los vertebrados terrestres, aunque el grado de desarrollo del epitelio olfativo y en particular de los bulbos olfativos, varía sustancialmente entre los distintos órdenes, familias y géneros de aves. Estas diferencias podrían demostrar el uso diferencial de los diferentes sentidos (olfato, visión y oído) como canales principales para obtener información en los diferentes grupos. El tamaño y la complejidad del sistema olfativo son mayores en aquellas aves que anidan en el suelo, que viven

asociadas al agua, con reproducción colonial, y son carnívoras o piscívoras. Sin embargo, el significado funcional del tamaño del bulbo olfativo no está claro: una especie con un bulbo relativamente pequeño puede detectar olores tan eficazmente como otra con el sistema olfativo mucho más desarrollado. Éste es el caso de las gallinas (*Gallus gallus*). Fluck *et al*. (1996) demostraron que las gallinas discriminaban entre el olor de los gatos (*Felis catus*), que provocaba una respuesta de miedo, y olores control. Sin embargo, apenas hay trabajos acerca del aislamiento de tales sustancias. Una excepción la constituye el trabajo de Mason y colaboradores (1991), que mostró que la ortoaminoacetofenona, presente en las glándulas olorosas de los mustélidos, provoca una reacción de miedo en los estorninos (*Sturnus vulgaris*).

Clase Mamíferos

La mayoría de los mamíferos son nocturnos y viven en ambientes físicamente complejos. En estas circunstancias la transmisión de información se realiza mayoritariamente por el canal químico (Kleiman 1966; Ralls 1971; Johnson 1973; Halpin 1986).

En mamíferos se han propuesto varios compuestos como desencadenantes de respuestas de miedo. Uno de ellos es el 2,5-dihidro-2,4,5-trimetiltiazolina (TMT), presente en las heces de zorro (Vernet-Maury 1980). Se han realizado numerosos experimentos utilizando este compuesto como repelente (Burwash *et al*. 1998 a,b; Fendt *et al*. 2003; Endres *et al*. 2005), aunque los resultados sugieren que no todas las especies responden a este olor (Arnould *et al*. 1998). En ratas negras (*Rattus rattus*) en condiciones de laboratorio, el 3,3-dimetil-1,2-ditiolano (DMDIT), que se encuentra en las secreciones de las glándulas anales de los mustélidos (*Mustela* sp.) produjo resultados similares a los provocados por el TMT (Burwash *et al*. 1998a), pero en condiciones naturales ninguno de los dos produjo una respuesta clara en las ratas (Burwash *et al*. 1998b). Arnould y colaboradores (1998) observaron que ciertos ácidos grasos y un compuesto sulfurado presentes en los excrementos de perro, repelían a las ovejas. Los autores sugirieron que los ácidos grasos podrían estar enmascarando otras sustancias presentes en concentraciones mucho más pequeñas. En general, se cree que los compuestos sulfurosos resultantes del metabolismo de la carne, serían los responsables de un olor no general de carnívoro, reconocible por las presa (Nolte *et al*. 1994). Sin embargo, en algunas secreciones o excreciones de ciertos carnívoros, los compuestos sulfurosos son escasos o están ausentes. Por ejemplo la orina de los hurones

(*Mustela furo*) carece de compuestos sulfurosos, y la quinolina, una de las sustancias volátiles contenidas en su orina, provoca comportamiento de huída en ratones (*Mus musculus*) (Zhang *et al*. 2007).

En resumen, a pesar de que se han realizado muchos trabajos en los que se ha intentado identificar qué sustancias desencadenan las respuestas antidepredatorias, la información obtenida es muy variada. Sin embargo, lo que sí se ha puesto de manifiesto es que no existe una clave única, ni siquiera dentro de una clase taxonómica. Se pueden distinguir dos grandes grupos entre las especies presa: el de aquéllas que producen sustancias de alarma y las reconocen en las excreciones de sus depredadores, y el de las que detectan sustancias resultantes del metabolismo de la dieta. Dado que (1) diferentes especies desarrollan respuestas de miedo ante sustancias distintas y (2) las señales de alarma se encuentran en grupos muy diversos en células análogas, parece que el mecanismo de reconocimiento de los depredadores ha aparecido en numerosas ocasiones a lo largo de la historia evolutiva.

2. Valoración del riesgo de depredación

Los animales utilizan claves tanto directas como indirectas para evaluar el riesgo de depredación. Las claves directas del riesgo de depredación serían la presencia física del depredador o la observación de un ataque. Las claves indirectas son aquéllas que confieren a diferentes microhábitats o momentos, diferentes riesgos de depredación. Por ejemplo, las variaciones en la intensidad de iluminación modifican el comportamiento de muchos roedores. En las noches de luna llena los micromamíferos reducen su actividad global o la restringen a los microhábitats con mayor cobertura vegetal, debido a que en estas noches los animales resultan más conspicuos para sus depredadores (Dickman 1992; Kotler *et al*. 2002). Los animales consideran más seguros ciertos hábitats según el tipo de depredadores a los que se enfrentan (Moreno *et al*. 1996; Woodley y Peterson 2003; Amo *et al*. 2004; Powell y Banks 2004). Por ejemplo, muchos pequeños mamíferos consiguen no ser detectados por sus depredadores aéreos si se encuentran en zonas con mucha cobertura vegetal, mientras que ese tipo de hábitats son más peligrosos frente a los depredadores terrestres. Así lo demostraron Moreno y colaboradores (1996) en un trabajo realizado con conejos. Los conejos durante el día, cuando su peligro principal son los depredadores aéreos, se encuentran preferentemente en lugares con

mucha cobertura vegetal, mientras que durante la noche, abandonan esa cobertura a favor de zonas más abiertas. Otra clave indirecta es la precipitación, ya que suele reducir la habilidad de los depredadores para detectar a sus presas y éstas se encuentran más activas (Orrock *et al*. 2004).

Independientemente del tipo de información de la que dependan, la intensidad de la respuesta antidepredatoria de un animal debería estar en concordancia con el riesgo de depredación al que está sometido. Los comportamientos antidepredatorios suelen ser costosos, ya que los animales se enfrentan a situaciones de compromiso en términos de tiempo y energía que pueden influir en su éxito biológico (Helfman 1989). Por lo tanto los animales tienen que valorar el riesgo al que están expuestos y reaccionar en consecuencia (Helfman 1989; Loose y Dawidowicz 1994; Puttlitz *et al*. 1999; Amo *et al*. 2004; Kusch *et al*. 2004; Foam *et al* 2005; Teplitsky *et al*. 2005; Ferrari y Chivers 2006; Ferrari *et al*. 2006; Zhao *et al*. 2006; Kesavaraju *et al*. 2007). Es lo que se conoce como la hipótesis de la respuesta dependiente del riesgo de depredación (*threat-sensitive predator hypothesis*). Por ejemplo, Horat y Semlitsch (1994) demostraron que dos especies de rana, *Rana lessonae* y *Rana esculenta*, variaban su comportamiento de acuerdo al riesgo potencial de depredación al que estaban sometidas. En las larvas del mosquito *Ochlerotatus triseriatus*, diferentes concentraciones del olor de uno de sus depredadores provocaba modificaciones graduales en su comportamiento (Kesavaraju *et al*. 2007). Sin lugar a dudas el grupo más utilizado en el estudio de la adquisición de información y valoración del riesgo de depredación ha sido el de los peces, que han mostrado también los resultados más sofisticados (Chivers *et al*. 2001; Brown *et al*. 2004; Ferrari *et al*. 2006). Algunos peces pueden determinar el tamaño de sus depredadores a partir de su olor (Kusch *et al*. 2004), e incluso evaluar la distancia a la que se encuentran y su densidad relativa (Ferrari *et al*. 2006).

3. Estrategias antidepredatorias

Una vez que los animales reconocen la presencia de un depredador y estiman que el riesgo es lo suficientemente elevado, desarrollan una respuesta antidepredatoria con objeto de reducir la probabilidad de ser depredados. Las estrategias para reducir el riesgo de depredación son muy diversas y afectan a diferentes estadíos de la secuencia de la depredación (ver tabla 1). El tipo de estrategia que un animal empleará va a depender de

su modo de vida, del riesgo de depredación al que esté sometido, y de los estilos comportamentales que muestre (Gosling 2001; Sih *et al.* 2004).

Modificaciones en los historiales de vida

La presencia de depredadores modifica ciertas características de la vida de algunos organismos. La mayoría de los ejemplos tienen que ver con el tamaño de las puestas, la edad a la que los huevos eclosionan, modificaciones en la fecundidad o incluso cambios relacionados con la estación en la que se reproducen (Magnhagen 1991; Scheuerlein *et al*. 2001). Algunas de las estrategias que normalmente se clasifican como modificaciones en los historiales de vida tienen su origen en modificaciones fisiológicas o comportamentales. Por ejemplo, se sabe que la presencia de depredadores hace que las larvas de libélula (*Coenagrion hastulatum*) crezcan menos. Esto se debe a que las larvas reducen su actividad y por lo tanto dedican menos tiempo a la alimentación (Brodin *et al*. 2006).

Independientemente del origen de la modificación, lo que está claro es que este tipo de estrategias son costosas y suponen consecuencias a medio o largo plazo en el éxito biológico, en la distribución de la especie, en la dinámica de poblaciones e incluso en la estructura de las comunidades ecológicas. De hecho, los efectos indirectos de los depredadores pueden tener efectos mayores en las poblaciones de las especies presa que los efectos directos (la propia depredación) (Lima 1998 a; Preisser *et al*. 2005, Luttbeg y Kerby 2005).

Algunos ejemplos de modificaciones en los historiales de vida proceden de los roedores. Por ejemplo, los topillos rojos (*Clethrionomys glareolus*) reducen la inversión en la reproducción cuando están sometidos a una presión de depredación elevada (Korpimäki *et al*. 1994; Ylönen y Ronkainen 1994). El olor de armiños (*Mustela erminea*) y comadrejas (*Mustela nivalis nivalis*) provoca la supresión de la reproducción de los topillos agrestes, *Microtus agrestis* (Koskela e Ylönen 1994), mientras que en los topillos árticos (*Clethrionomys rutilus*) la presencia de depredadores provoca una reducción del tamaño de las crías, con las consiguientes consecuencias para su éxito biológico (Heikkilä *et al*. 1993).

En anfibios, la principal estrategia que afecta a los historiales de vida está relacionada con la metamorfosis. En general, los anfibios que se encuentran bajo riesgo de depredación se metamorfosean más tarde, y cuando lo hacen son de mayor tamaño que los animales control (revisado por Relyea 2007). Los embriones de *Rana clamitans*

sometidos a riesgo de depredación por parte de depredadores especialistas en huevos, eclosionan con un tamaño pequeño y en un estadío de desarrollo temprano. Sin embargo, si el riesgo proviene de depredadores de larvas eclosionan más tarde, con un tamaño mayor y en un estadío más avanzado (Ireland *et al*. 2007).

Modificaciones fisiológicas

La producción de toxinas constituye un mecanismo fisiológico que permite evitar en cierta medida ser comido. Es el caso de muchos anuros, que producen toxinas eficaces contra muchos de sus depredadores potenciales, tales como la bufotoxina (Flier *et al*. 1980). Otros animales resultan repulsivos debido a ciertas secreciones glandulares. Por ejemplo, las musarañas poseen glándulas ventrales y dorsales que producen un olor repulsivo para la mayoría de depredadores. Se ha sugerido que este olor puede advertir de la posesión de una saliva venenosa (Macdonald 1977). Otro ejemplo de sustancias hediondas serían las secreciones de los sacos anales de las mofetas (Wood *et al.* 2002).

Otro tipo de adaptación fisiológica es la resistencia que muestran algunas especies de mamíferos pequeños al veneno de serpientes simpátricas. Estos mamíferos poseen factores antihemorrágicos en el suero que les hacen resistentes a múltiples mordeduras (Wit 1982, Wit y Westrom 1987).

Modificaciones morfológicas

Las defensas morfológicas intervienen sobre todo en las primeras y últimas etapas de la depredación. Las coloraciones crípticas y el enmascaramiento permiten que el animal pase inadvertido, mientras que otros tipos de modificaciones morfológicas, tales como el desarrollo de espinas o de morfologías inducidas por la presencia de depredadores, evitan o disminuyen el consumo de esas especies.

Se dice que un animal es críptico si se parece a una muestra aleatoria del ambiente en el momento y en el microhábitat en el que resultaría más vulnerable frente a un depredador visual (Endler 1978). Probablemente la cripsis sea la estrategia antidepredatoria más extendida entre los animales homeotermos. La cripsis se consigue de las siguientes maneras:

1) pareciéndose al entorno mediante un pelaje uniforme o con un patrón de rayas o líneas que se mezcle con el ambiente en el que el animal habita.

2) minimizando la sombra mediante una pigmentación oscura en la parte dorsal y clara en la ventral, de tal manera que, cuando la luz incide verticalmente, se reduce la cantidad de sombra que su cuerpo produce (Kiltie 1989)

3) mediante una coloración disruptiva, que desdibuja la forma del animal; se consigue con patrones de tonos y colores contrastados, como la banda negra lateral de las gacelas de Thomson, *Gazella thomsoni* (Caro 2005).

Otro tipo de estrategia son las variaciones morfológicas inducidas por depredadores. Las carpas (*Carassius carassius*) que viven en lugares con peces piscívoros desarrollan una defensa morfológica que consiste en tener cuerpos anchos. Esto reduce la eficacia de sus depredadores piscívoros que están limitados por la abertura de la boca. Cuando no hay depredadores la morfología es más aplanada (Nilsson *et al* 1995). Una estrategia más elaborada sería la presencia de espinas o dientes en aquellas poblaciones con un alto riesgo de depredación. Esto sucede sobre todo en invertebrados acuáticos, como por ejemplo, diversas especies del género *Daphnia* recurren a las espinas como modo de defensa (Preisser *et al*. 2005).

Los equidnas, tenrecs, erizos y puercoespines presentan púas que les protegen de los depredadores. Todos poseen además otros tipos de comportamientos antidepredatorios. Por lo general utilizan refugios y reducen la actividad como primera medida, mientras que suelen recurrir a las espinas como último recurso (Caro 2005). Otras especies de mamíferos, como los pangolines, poseen escamas epidérmicas que son eficaces frente a muchos de sus depredadores.

Otra estrategia morfológica ampliamente distribuida es el mimetismo. En el mimetismo mülleriano, dos o más especies nocivas, peligrosas o de sabor desagradable comparten una señal similar. En insectos este tipo de mimetismo es muy común pero en vertebrados es más difícil de encontrar. Un ejemplo es el que ofrecen las ranas venenosas (Dendrobátidos: *Dendrobates imitator* y *Dendrobates variabilis*) (Symula *et al*. 2001) o las serpientes de coral (*Micruroides* sp., *Micrurus* sp., *Lampropeltis* sp.) (Greene y McDiarmid 1981). En el mimetismo batesiano, una especie inocua se parece a una nociva, peligrosa o de sabor desagradable (Caro 2005). El enmascaramiento, al igual que el mimetismo, reduce la probabilidad de identificación por parte de los depredadores. Consiste en parecerse a objetos no comestibles, como partes de plantas o piedras.

Modificaciones comportamentales

De todos los mecanismos existentes para reducir el riesgo de depredación, las estrategias comportamentales han sido las más estudiadas (ver tabla 1). En primer lugar se van a comentar las principales modificaciones en el comportamiento en respuesta a depredadores. Haremos especial hincapié en los tipos de estudios que se han considerado para la elaboración de la tabla 1. Deberíamos aclarar que el objeto de la tabla 1 es otra aportación a las numerosas revisiones que se han realizado sobre este tema (p.e. Lima y Dill 1990; Kats y Dill 1998; Lima 1998 b; Apfelbach *et al*. 2005), y su principal función es mostrar los tipos de estrategias que emplean los animales en las diferentes etapas de la secuencia de la depredación.

Patrones temporales-espaciales en la actividad. En este apartado se incluyen tanto los cambios temporales como los espaciales. Fenn y Macdonald (1995) detectaron un cambio temporal en la actividad de una población de ratas (*Rattus norvegicus*) sometidas a una presión alta de depredación. Éstas se volvían mayoritariamente diurnas, pero tras la eliminación de los depredadores, volvieron a su ritmo nictimeral. Otra estrategia muy común es el cambio espacial de la actividad. Los animales utilizan zonas que consideran más seguras, ya sea por la presencia de cobertura vegetal (Moreno *et al*. 1996; Creel *et al*. 2005) o se alejan de los olores de depredadores (Grostal y Dicke 2000). No se han incluido aquellos trabajos en los que la metodología sólo permite detectar cambios instantáneos en el comportamiento, como los que han utilizado trampas con olores (Stoddart 1976; Stoddart 1982; Dickman 1992; Tobin *et al*. 1995).

Una estrategia antidepredatoria mixta es la que siguen muchos invertebrados acuáticos, que realizan movimientos en la columna de agua con un ritmo determinado (migraciones verticales circadianas). Los cambios en la actividad serían tanto espaciales como temporales, ya que por el día se encuentran en las capas más profundas de la columna de agua, segregados espacialmente de los depredadores visuales que se encuentran en las capas más superficiales, y por la noche aprovechan las capas superiores, que son más ricas en nutrientes (Loose y Dawidowicz 1994).

Aumento de la vigilancia. Es otro de los comportamientos más extendidos y también de los más estudiados. En la tabla 1 sólo se han incluido los trabajos que mostraron un aumento de la vigilancia como consecuencia de un encuentro con un depredador, real o simulado. Muchos otros trabajos han recurrido a las tasas basales de vigilancia como un

modo de estudiar el efecto del grupo en la vigilancia, pero aquí no se han tenido en cuenta (tabla 1) (Elgar 1989; Pravosudov y Grubb 1999; Childress y Lung 2003; Cameron y du Toit 2005).

Reducción de la actividad. Los animales aumentan su probabilidad de sobrevivir si reducen su actividad en aquellos momentos en los que el riesgo de depredación es mayor (Lima y Dill 1990; Ylönen y Ronkainen 1994; Norrdahl y Korpimäki 1998). Ante un riesgo potencial de depredación muchos animales optan por dejar de realizar cualquier actividad que suponga estar más expuesto a los depredadores. Un tipo especial de reducción de la actividad sería el uso de refugios. Las consecuencias son similares, ya que en ambos casos el tiempo durante el cual están inactivos supone un coste para el individuo en términos de pérdida de oportunidades alimentarias, reproductivas, etc. Por lo tanto, los animales deben tomar decisiones relacionadas con la elección del mejor momento para abandonar los refugios o la inactividad (Martín y López 1999).

Agregación. Se cree que una de las razones por las que los animales viven en grupos es defenderse de los depredadores. La presencia de un mayor número de individuos permitirá detectar antes la presencia de un depredador (many eyes hypothesis Bertram 1980). El efecto de confusión es otra posible ventaja de la agregación: la presencia de muchos individuos hace que los depredadores necesiten más tiempo para elegir y capturar a una presa (Milinski 1977; Schradin 2000). Los movimientos erráticos o en zig-zag de la presa durante la huída generan confusión en el depredador. Incluso en ausencia de este tipo de movimientos, en muchas ocasiones, el comportamiento del resto de los congéneres, que se cruzan continuamente en la trayectoria entre el depredador y la presa, dificulta el que un depredador siga a una única presa (Caro 2005). Otra de las ventajas de la vida en grupo podría ser la reducción del riesgo individual (efecto de dilución: Hamilton 1971) siendo especialmente efectivo ante ataques de depredadores oportunistas (Cresswell *et al*. 2003). Sin embargo, la posición dentro del grupo constituye un elemento clave del riesgo individual de depredación (Di Blanco y Hirsch 2006). Los animales que se encuentran en la periferia del grupo están en una situación de riesgo mayor que los que están en el interior (Hamilton 1971).

Señales disuasorias de la persecución. Las presas adoptan posturas cuya función es informar al depredador de (a) que ha sido detectado y/o (b) de su habilidad para escapar. La intención es desalentar al depredador, ya que la probabilidad de que pueda alcanzar y

subyugar a una presa que lo ha detectado es pequeña. Estas señales pueden ser más o menos costosas dependiendo de que transmitan información sobre la calidad de la presa (señales zahavianas) o simplemente comuniquen al depredador que ha sido detectado (FitzGibbon 1989, Scannell *et al*. 2001).

Acoso a los depredadores (mobbing). Suele ser característico de animales que viven en grupos. Incluye tanto las vocalizaciones destinadas a asustar o a amenazar, que también sirven como señales de alarma a congéneres, como la observación, la aproximación e incluso la agresión al depredador.

Por regla general los animales no utilizan un solo tipo de táctica para evitar la depredación, y dentro de cada categoría pueden emplear diferentes tipos. Por ejemplo, un animal puede tener coloración críptica (defensa morfológica) y al mismo tiempo mostrar una tasa alta de vigilancia y modificar su uso del espacio (modificaciones comportamentales). Las distintas poblaciones de la misma especie seguirán estrategias comportamentales diferentes, que estarán en gran medida determinadas por la complejidad estructural de sus hábitats naturales (Brown y Warburton 1997). Además, el tipo de depredador puede ser determinante a la hora de elegir una estrategia. Por ejemplo, Cresswell y colaboradores (2003) concluyeron que ante un depredador oportunista, la mejor estrategia de la presa sería permanecer en un grupo grande (efecto de dilución), mientras que ante un depredador al acecho, el incremento en el nivel de vigilancia sería la clave para escapar con éxito de depredadores. Por lo tanto, no todas las poblaciones están sometidas al mismo riesgo depredación y dentro de una población, no todos los individuos de una especie tienen el mismo riesgo de ser depredados.

Riesgo diferencial de depredación

El riesgo individual de depredación depende de factores estacionales, sexuales, y ontogénicos, entre otros (Borowski 2002):

Uno de los factores que influye en la depredación diferencial es la movilidad del individuo. Norrdahl y Korpimäki (1998) demostraron que los depredadores terrestres de los topillos (*Microtus agrestis* y *Microtus rossiaemeridionalis*), que en el área de estudio eran principalmente pequeños mustélidos (*Mustela erminea* y *Mustela nivalis*), depredaron selectivamente sobre los menos móviles. Similares resultados se obtuvieron en las ratas canguro (*Dipodomys merriami*) (Daly *et al*. 1990). En ocasiones, la mayor

movilidad la muestra sólo uno de los sexos, que suele ser el que durante la época reproductiva busca pareja activamente (Forsgren y Magnhagen 1993).

Sin embargo, la reducción de la movilidad también puede hacer que uno de los sexos sufra una mayor depredación. Es lo que les sucede a las hembras grávidas, cuya velocidad de escape es menor, o las hembras que acotan sus movimientos a las proximidades de la madriguera de cría (Norrdahl y Korpimäki 1998).

Otro factor que afecta a la depredación diferencial es la tasa individual de vigilancia. Los animales que detecten a los depredadores directamente tendrán una probabilidad de escapar con éxito mayor que los que utilizan el comportamiento de los otros individuos del grupo para detectar a los depredadores (Bednekoff y Lima 1998). Los depredadores que cazan al acecho seleccionan aquellas presas que vigilan menos (Cresswell *et al*. 2003). Por ejemplo, en las gacelas son los machos los que sufren una mayor depredación, mientras que las hembras suelen mostrar tasas de vigilancia mayores (FitzGibbon 1990). De manera similar, en los ciervos (*Cervus elaphus*) los machos vigilan menos (Winnie y Creel 2007). Por el contrario, en los perritos de las praderas de cola negra (*Cynomys ludovicianus*) son los machos los que muestran una tasa de vigilancia mayor (Loughry 1993). Asimismo, los animales en peor condición física maximizan la ingesta energética en contraposición a otras actividades, especialmente la vigilancia (FitzGibbon 1989; Murray 2002) y por lo tanto, corren un riesgo mayor de ser depredados.

En aquellos animales que se agrupan para realizar alguna de sus actividades, como comer o desplazarse, un factor determinante del riesgo de depredación individual es la posición dentro del grupo (Hamilton 1971). Los depredadores a menudo atacan a los individuos aislados o que se encuentran en la periferia del grupo. Los animales en estas posiciones desfavorables suelen tener una tasa de vigilancia mayor. En coatíes (*Nasua nasua*), los individuos que vigilan más son los que están en la periferia y situados en la zona de avance del grupo, ya que la probabilidad de encuentro con depredadores y por lo tanto el riesgo al que se enfrentan en esa posición es máxima (Di Blanco y Hirsch 2006).

Dentro de un grupo, los individuos juveniles sufren más depredación que los adultos, debido al rango más amplio de depredadores potenciales (Parer 1977) y a la menor experiencia en el trato con los depredadores (Vitale 1989).

A modo de resumen, se podría decir que los animales reconocen a sus depredadores, evalúan su riesgo, y desarrollan una respuesta antidepredatoria. Probablemente la estrategia más segura para la presa sería detectar a los depredadores antes de que los depredadores la detecten a ella y desarrollar una respuesta antidepredatoria adecuada. Si son capaces de esto, podrían resolver el conflicto en las primeras etapas de la secuencia depredatoria, cuando la probabilidad de éxito es mayor. Además evitarían los encuentros directos, que son tan peligrosos para la mayoría de las especies presa.

El reconocimiento de los depredadores por el olor y las respuestas subsiguientes han originado numerosos trabajos (revisados por Kats y Dill 1998; Lima 1998 b; Apfelbach *et al*. 2005).

4. Cuestiones sin resolver

A pesar de los muchos estudios que se han llevado a cabo sobre el comportamiento antidepredatorio, algunas preguntas siguen sin contestación. Vamos a destacar tres, que son las que han desencadenado la elaboración del trabajo que en esta memoria se va a presentar.

Una de las cuestiones es si el comportamiento está genéticamente modulado o, si por el contrario, es aprendido. Si fuese innato, las poblaciones que llevan mucho tiempo sin estar en contacto con sus depredadores, ya sea por extinciones locales o totales, desarrollarían un comportamiento antidepredatorio similar al de las poblaciones de la misma especie sometidas a un riesgo de depredación real. Berger y colaboradores (2001) llevaron a cabo un estudio en el que compararon el comportamiento antidepredatorio de alces (*Alces alces*) que llevaban aproximadamente cien años sin depredadores con el comportamiento de alces que sí que estaban en contacto con depredadores (lobos *Canis lupus* y osos *Ursus arctos*). Los resultados obtenidos apuntaban a que los alces que no estaban familiarizados con los depredadores exhibían una respuesta mucho menor que los animales simpátricos con dichos depredadores. Similares resultados se han encontrado en algunos marsupiales (*Macropus eugenii* y *Thylogale thetis*), en los que la ausencia de depredadores simpátricos ha provocado que no reconozcan a sus depredadores por el olor (Griffin *et al*. 2000; Blumstein *et al*. 2002). En iguanas marinas sin experiencia previa con depredadores, la persecución por parte de un depredador potencial no activaba la respuesta fisiológica de estrés. Sólo se activaba

una vez que habían sufrido una primera captura y una persecución subsiguiente (Rödl *et al*. 2007).

En otras especies, los resultados obtenidos son los opuestos. En los caracoles acuáticos (*Physa acuta*) el reconocimiento químico de los depredadores es innato (Turner *et al*. 2006). Muchos roedores también exhiben un comportamiento antidepredatorio independiente de la experiencia previa (Calder y Gorman 1991; Barreto y Macdonald 1999; Fendt 2006).

Como regla general, se puede decir que la desaparición de alguna o de todas las respuestas comportamentales se produce si el coste que ocasiona su mantenimiento supera los posibles beneficios (Blumstein 2002). Sin embargo, la falta total de estrategias antidepredatorias hacia un depredador arquetípico no es corriente en sistemas terrestres continentales, ya que no suelen darse las condiciones necesarias (ausencia total de depredadores) (Cox y Lima 2007).

Por lo tanto, nos planteamos hasta qué punto la experiencia previa con depredadores juega un papel esencial en su reconocimiento y en las respuestas desarrolladas ante los mismos (ver más adelante en el apartado de Objetivos).

Otro tema acerca del cual no hay consenso es el de la validez de los experimentos de laboratorio sobre la detección y respuesta a los depredadores por el olor (Kavaliers y Choleris 2001; Wolff 2003). La mayoría de los trabajos se han realizado en condiciones de laboratorio y no todos los resultados obtenidos se han podido validar en condiciones naturales (Burwash *et al*. 1998 a y b). Por ejemplo, en el laboratorio, los topillos evitaban las zonas en las que había olor de depredador o reducían la ingesta de alimento (Jedrzejewski *et al*. 1993), mientras que en condiciones naturales esto no se pudo corroborar (Pusenius y Ostfeld 2002). Algunos autores han sugerido que las reacciones observadas en el laboratorio podrían ser artefactos, puesto que los animales están en un ambiente que podrían percibir como peligroso y las respuestas se exageran (Sapolsky 1992; Boissy 1995; Magurran *et al*. 1996).

Por todo lo expuesto, sería deseable que los experimentos de laboratorio se validaran en condiciones naturales. Esto sería particularmente importante en las especies sociales, debido a que el apoyo del resto de individuos del grupo podría atenuar las respuestas a los depredadores observadas en el laboratorio. Además, en condiciones naturales los animales tienen otros compromisos vitales, tales como cubrir las demandas energéticas, y como han señalado algunos autores, los animales que están en peor

condición física anteponen el tiempo que deben dedicar a la alimentación al desarrollo de las respuestas antidepredatorias (FitzGibbon 1989; Murray 2002).

Finalmente, otra cuestión apenas explorada es el desarrollo de las respuestas fisiológicas de estrés provocadas por la presencia del olor de depredadores. Se sabe que la percepción de diferentes agentes estresantes provoca la activación del sistema simpáticoadrenomedular y del eje hipotalámico-pituitario-adrenal (HPA) (von Holst 1998; Matteri *et al*. 2001; Möstl y Palme 2002). El resultado es un aumento de los niveles de catecolaminas y glucocorticoides cuya función es movilizar y redirigir la energía hacia actividades relacionadas con la respuesta de "lucha o huída" (Sapolsky 1992; von Holst 1998; Buchanan 2000; Creel 2001). Aunque el mecanismo está muy estudiado, pocas veces se ha considerado en estudios sobre el reconocimiento de los depredadores por el olor (Vernet-Maury *et al*. 1984). Los ataques de depredadores, o la percepción del riesgo de depredación son eventos incontrolables e impredecibles, dos condiciones suficientes para provocar una respuesta fisiológica de estrés (Boissy 1995; Landys *et al*. 2006). El hecho de que un animal no desarrolle una respuesta antidepredatoria, no significa que no detecte la presencia de depredadores (Ydenberg y Dill 1986). Sin embargo, las respuestas fisiológicas se suelen conservar, aunque no siempre se traduzcan en una respuesta comportamental (Eilam *et al*. 1999). Por lo tanto, la cuantificación de la respuesta de estrés podría constituir una herramienta importante en aquellos casos en los que las especies no tienen experiencia previa con depredadores.

Las circunstancias que se acaban de describir han sido las desencadenantes de la realización de la presente tesis doctoral. Nos centramos en el reconocimiento de los depredadores por el olor y analizamos si los conejos sin experiencia previa con depredadores discriminaban entre el olor de un depredador y de un no depredador, tanto en condiciones de laboratorio como en semilibertad. En ambos casos medimos las respuestas comportamentales y fisiológicas.

5. El modelo animal

La especie objeto de estudio ha sido el conejo (*Oryctolagus cuniculus*). El conejo constituye un buen modelo para realizar un trabajo sobre el comportamiento provocado por el olor de los depredadores por los siguientes motivos:

1) Se sabe que los conejos utilizan en gran medida el canal químico para el intercambio de información inter e intraespecífica (Mykytowycz 1968; Mykytowycz y Gambale 1969; Bell 1980; Sneddon 1991).
2) El conejo es una de las presas principales de gran número de mamíferos terrestres, aves y reptiles (Delibes e Hiraldo 1981; Jaksic y Soriguer 1981) y, por lo tanto, su tasa de encuentro con excreciones y secreciones de sus depredadores debería ser alta.
3) Se trata de una especie social en la que se pueden analizar los costes y los beneficios de la pertenencia a un grupo. Por un lado se sabe que los lazos sociales podrían reducir el efecto de los agentes estresantes, como podría ser un depredador, y por otra parte, los animales dentro de un grupo tienen que controlar la posición y el riesgo potencial que suponen los mismos miembros del grupo. Por lo tanto, los animales se enfrentan a una situación de compromiso entre vigilar a los depredadores y evitar o incitar agresiones.
4) No existen trabajos previos, excepto el realizado por Boag y Mlotkiewicz (1994), en el que se centraron en el conejo como plaga agrícola.

6. Objetivos

Los objetivos que se pretenden cubrir son los siguientes:
(1) Determinar si los conejos reconocen a los depredadores por el olor.
(2) Averiguar si las respuestas comportamentales y fisiológicas al olor de depredadores están genéticamente moduladas.
(3) Analizar el efecto de la edad y del sexo de los individuos en la respuesta antidepredatoria.
(4) Analizar el principal comportamiento antidepredatorio del conejo, la vigilancia, en diferentes contextos (sociales y presencia de depredadores).
(5) Validar una técnica no invasiva para la determinación de la respuesta fisiológica de estrés de los conejos.

7. Hipótesis de partida

1) El reconocimiento de los depredadores será innato, puesto que el aprendizaje supondría un riesgo muy elevado una vez que se produce un encuentro directo con un depredador. Sin embargo, no descartamos que la experiencia juegue un papel clave en la

intensidad de la respuesta. Dadas las capacidades sensoriales de los conejos, esperamos encontrar un reconocimiento de los depredadores por el olor.

2) Dado que tienen un rango amplio de depredadores suponemos que el comportamiento antidepredatorio exhibido será general, permitiéndoles así responder a las diferentes especies que depredan sobre el conejo.

3) Los animales juveniles responderán de una manera menos específica que los adultos (respuesta cuantitativa Inglis 1979), y suponemos que en la época reproductiva las diferencias entre machos y hembras se maximizarán debido a que, en esa época, los diferentes sexos tendrán diferentes prioridades.

8. Estructura del trabajo

El trabajo que se presenta está organizado en una serie de experimentos diseñados para responder a los objetivos propuestos. Estos experimentos han dado lugar a una serie de artículos, a los que nos referiremos a continuación:

El primer artículo es el resultado de un experimento que llevamos a cabo para determinar en condiciones de laboratorio si conejos que nunca habían estado en contacto con carnívoros podían reconocer a los depredadores por el olor y qué respuestas desarrollaban. Se tuvieron en cuenta tanto las respuestas comportamentales, como cambios en la ingesta de alimento, en la actividad, en la vigilancia, como las respuestas fisiológicas.

En el segundo artículo nos planteamos comprobar si las respuestas obtenidas en condiciones de laboratorio se mantenían en condiciones de semilibertad, teniendo en cuenta que los factores sociales y energéticos podrían jugar un papel importante como moduladores de las respuestas antidepredatorias. Además, se comparó el comportamiento de los individuos juveniles y adultos para analizar la ontogenia del comportamiento antidepredatorio.

El tercer artículo se centró en el comportamiento antidepredatorio principal del conejo: la vigilancia. Algunas observaciones previas apuntaban a que los conejos poseen dos formas diferentes de vigilancia, una de alta intensidad y otra de baja intensidad y se planteó determinar si los diferentes comportamientos respondían a diferentes estímulos (estímulos sociales y presencia de depredadores).

El objetivo del cuarto artículo fue validar una prueba no invasiva para medir glucocorticoides en heces. Los glucocorticoides son secretados en respuesta a los

agentes estresantes y son una medida muy utilizada para valorar el estado de un animal. En muchas ocasiones es inviable capturar a los animales, por lo no se pueden tomar muestras de sangre. Además, las medidas repetidas en animales pequeños tampoco son posibles, por lo que es aconsejable recurrir a otro tipo de técnicas. Con este motivo se analizaron los metabolitos de los glucocorticoides en las heces. Debido a que las rutas metabólicas de la excreción de los glucocorticoides son específicas, todo inmunoensayo enzimático debe validarse tanto fisiológica como biológicamente para cada especie. Para ello diseñamos un experimento en el que los conejos se enfrentaron a la presencia de depredadores simulados y además se sometieron a una inyección de ACTH.

Por último, y casi a modo de anexo, se presenta un trabajo en el que los conocimientos adquiridos se aplican a un caso concreto: el uso de un agente estresante conocido (el olor de zorro) para determinar la consistencia de los estilos de comportamiento de los conejos.

Tabla 1- *Estrategias antidepredatorias*

Estadío	**Estrategias antidepredatorias**		**Especie**	**Fuente**
Encuentro	Cambio en la actividad	Temporal	*Daphnia magna*	Loose y Dawidowicz 1994
			Tursiops aduncus	Heithaus y Dill 2002
			Gerbillus andersoni	Kotler *et al*. 2002
			Rattus norvegicus	Fenn y Macdonald 1995
		Espacial	*Neoseiulus cucumelis*	Magalhães *et al*. 2005
			Tetranychus urticae	Grostal y Dicke 2000
			Ochlerotatus triseriatus	Kesavaraju *et al*. 2007
			Bufo boreas	Kiesecker *et al*. 1996
			Bufo cognatus	Flowers y Graves 1997
			Bufo microscaphus	
			Plethodon cinereus	Murray y Jenkins 1999
			Gambusia affinis	Smith y Belk 2001
			Zonotrichia leucophrys	Slotow y Rothstein 1995
			Melanotaenia eachamensis	Brown y Warburton 1997
			Papio cynocephalus	Cowlishaw 1997
			Acynonix jubatus	Durant 2000
			Tursiops aduncus	Heithaus y Dill 2002
			Cervus elaphus	Creel *et al*. 2005
			Rattus norvegicus	Hubbard *et al*. 2004
			Mus domesticus	Powell y Banks 2004
			Microtus oeconomus	Borowski 2002
			Clethrionomys glareolus	Jedrzejewski y Jedrzejewska 1990
				Jedrzejewski *et al*. 1993
			Peromyscus polionotus	Orrock y Danielson 2004
			Arvicola terrestris	Barreto y Macdonald 1999
			Oryctolagus cuniculus	Moreno et al. 1996
				Banks *et al*. 1999

	Selección del lugar de la oviposición	*Hyla squirella*	Binkley y Resetarits 2002
	Vigilancia	*Archocentrus nigrofasciatus* *Carduelis carduelis* *Corvus caurinus* *Platycercus elegans* *Petrogale xanthopus* *Dasypus novemcinctus* *Saguinus labiatus* *Nasua nasua* *Gazella thomsoni* *Marmota caudata* *Octodon degus* *Spermophilus columbianus* *Tamias striatus* *Sciurus carolinensis*	Foam *et al*. 2005 Glück 1987 Robinette y Ha 2001 Adams *et al*. 2006 Blumstein *et al*. 2001 McDonough y Loughry 1995 Caine y Weldon 1989 DiBlanco y Hirsch 2006 FitzGibbon 1989 Blumstein 1996 Ebensperger *et al*. 2006 Fairbanks y Dobson 2007 McAdam y Kramer 1998
Detección	Cortejo y cópula menos llamativos	*Syngnathus typhle* *Pomatoschistus minutus* *Poecilia reticulata*	Berglund 1993 Forsgren y Magnhagen 1993 Farr 1975
	Reducción de la actividad	*Carassius carassius* *Notophthalmus viridescens* *Rana lessonae* *Rana esculenta* *Rana temporaria* *Hyla regilla* *Bufo boreas* *Pseudemoia entrecasteauxii* *Meles meles* *Rattus rattus* *Rattus norvegicus* *Microtus socialis* *M. agrestis* *M. arvalis*	Petterson *et al*. 2000 Rohr y Madison 2001 Horat y Semlitsch 1994 Laurila 2000 Puttlitz *et al*. 1999 Kiesecker *et al*. 1996 Stapley 2004 Butler y Roper 1994 Burwash *et al*. 1998a Hubbard *et al*. 2004 Eilam *et al*. 1999 Gorman 1984

	Uso de refugio		*Physa acuta* *Uca lactea perplexa* *Chironomus rutilus* *Plethodon cinereus* *Bufo boreas* *Alces alces* *Rhombomys opimus* *Microtus agrestis* *Clethrionomys glareolus* *Spermophilus beldingi*	Turner *et al*. 2006 Jennions *et al*. 2003 Hölker y Stief 2005 Sullivan *et al*. 2002 Kiesecker *et al*. 1996 White y Berger 2001 Tchabovsky *et al*. 2001 Sundell e Ylönen 2004 Robinson 1980
	Confusión		*Daphnia magna* *Tenebrio molitor*	Milinski 1977 Schradin 2000
	Cripsis		*Perdix perdix* *Anolis stratulus* *Gazella thomsoni* *Peromyscus polionotus* *Sciurus carolinensis* *Spalax ehrenbergi* *Lepus arcticus*	Sage 1962 Ydenberg y Dill 1986 Caro 2005 Belk y Smith 1996 Heth *et al*. 1988 Gustafson y VanDruff 1990 Stoner *et al*. 2003
Identificación	Mimetismo	Batesiano	*Melampitta gigantea*	Dumbacher *et al*. 1992
		Mülleriano	*Dendrobates imitator*	Symula *et al*. 2001
	Enmascaramiento		*Rhipidura rufidorsa* *Myiobius atricaudus*	Hansell 2000

Acercamiento	Agregación	*Melanotaenia eachamensis* *Poecilia reticulata* *Culaea inconstans* *Carassius auratus* *Pimephales promelas* *Bufo cognatus* *Nasua narica* *Cervus elaphus* *Marmota marmota*	Brown y Warburton 1997 Kelley *et al*. 2003 Mathis y Smith 1993 Zhao *et al*. 2006 Ferrari y Chivers 2006 Kusch *et al*. 2004 Graves *et al*. 1993 Hass y Valenzuela 2002 Childress y Lung 2003 Blumstein *et al*. 1996
	Señales disuasorias de la persecución	*Psammodromus algirus* *Gazella thomsoni* *Odocoileus virginianus*	Martín y López 2001 FitzGibbon 1990 Bildstein 1983
Subyugación	Acoso (*mobbing*)	*Stegastes planifrons* *Xema sabini* *Aphelocoma coerulescens* *Dacelo novaeguineae* *Manorina melanocephala* *Dicrurus bracteatus* *Piliocolobus tephrosceles* *Nasua narica* *Odocoileus hemionus* *Spermophilus beecheyi* *Cynomys ludovicianus*	Helfman 1989 Stenhouse *et al*. 2005 Francis *et al*. 1989 Pavey y Smith 1998 Standford 2002 Hass y Valenzuela 2002 Lingle y Pellis 2002 Swaisgood *et al*. 1999 Loughry 1988
	Toxinas	*Pitohui dichrous* *Bufo spp.* *Phyllobates terribilis* *Mephitis mephitis*	Dumbacher *et al*. 1992 Flier *et al*. 1980 Daly *et al*. 1980 Lariviere y Messier 1996

	Resistencia al veneno	*Erinaceus europaeus* *Microtus ochrogaster* *Neotoma floridanus*	Wit y Westrom 1987 Wit 1982
	Espinas	*Daphnia carinata* *Daphnia ambigua*	Barry 1994 Hebert y Grewe 1985
	Otras morfologías defensivas	*Nucella lamellosa* *Daphnia pulex*	Appleton y Palmer 1988 Black 1993
Consumo	Digestión inocua	*Alvinia* sp.	Norton 1988

9. REFERENCIAS BIBLIOGRÁFICAS

Adams, J. L., Camelio, K. W., Trique, M. J. y Blumstein, D. T. 2006. Does information of predators influence general wariness? *Behavioral Ecology and Sociobiology*, **60**: 742–7 47.

Amo, L., López, P. y Martín, J. 2004. Wall lizards combine chemical and visual cues of ambush snake predators to avoid overestimating risk inside refuges. *Animal Behaviour*, **67**: 647–653.

Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A. y McGregor, I. S. 2005. The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neuroscience and Biobehavioral Reviews*, **29**: 1123–1144.

Appleton, R. D. y Palmer A. R. 1988. Water–borne stimuli released by predatory crabs and damaged prey induce more predator–resistant shells in a marine gastropod. *Proceedings of the National Academy of Science*, *USA*, **85**: 4387–4391.

Arnould, C., Malosse, C., Signoret, J. P. y Descoins, C. 1998. Which chemical constituents from dog feces are involved in its food repellent effect in sheep? *Journal of Chemical Ecology*, **24**: 559–576.

Banks, P. B., Hume, I. D. y Crowe, O. 1999. Behavioural, morphological and dietary response of rabbits to predation risk from foxes. *Oikos*, **85**: 247–256.

Barreto, G. R. y Macdonald, D. W. 1999. The response of water voles, *Arvicola terrestris*, to the odours of predators. *Animal Behaviour*, **57**: 1107–1112.

Barry, M. 1994. The costs of crest induction for *Daphnia carinata*. *Oecologia*, **97**: 278–288.

Bednekoff, P.A. y Lima, S.L. 1998. Re–examining safety in numbers: interactions between risk dilution and collective detection depend upon predator targeting behaviour. *Procceding of the Royal Society of London, B.*, **265**: 2021–2026.

Belk, M. C. y Smith M. H. 1996. Pelage coloration in oldfiel mice (*Peromyscus polionotus*): Antipredator adaptation? *Journal of Mammalogy*, **77**: 882–890.

Bell, D. J. 1980. Social olfaction in Lagomorphs. *Symposium of the Zoological Society London*, **45**: 141–164.

Berger, J., Swenson, J. E. y Persson, I.–L. 2001. Recolonizing carnivores and naïve prey: conservation lessons from pleistocene extinctions. *Science*, **291**: 1036–1039.

Berglund, A. 1993. Risky sex: male pipefishes mate at random in the presence of a predator. *Animal Behaviour*, **46**: 169–175.

Bertram B. C. R. 1980. Vigilance and group size in ostriches. *Animal Behaviour*, **28**: 278–286.

Bildstein, K. L. 1983. Why white–tailed deers flag their tails. *American Naturalist*, **121**: 709–715.

Binckley, C. A. y Resetarits Jr, W. J. 2002. Reproductive decisions made under threat of predation: squirrel treefrog (*Hyla squirella*) responses to banded sunfish (*Enneacanthus obesus*). *Oecologia*, **130**:157–161.

Black, A. R. 1993. Predator–induced phenotypic plasticity in *Daphnia pulex*: Life–history and morphological responses to *Notonecta* and *Chaoborus*. *Limnology and Oceanography*, **38**: 986–996.

Blumstein, D. T. 1996. How much does social group size influence golden marmot vigilance? *Behaviour*, **133**: 1133–1151.

Blumstein, D. T. 2002. Moving to suburbia: ontogenetic and evolutionary consequences of life on predator–free islands. *Journal of Biogeography*, **29**: 685–692.

Blumstein, D. T., Daniel, J. C. y Evans, C. S. 2001. Yellow–footed rock–wallaby group size effects reflect a trade–off. *Ethology*, **107**: 655–664.

Blumstein, D. T., Daniel, J. C., Griffin, A. S. y Evans, C. S. 2000. Insular tammar wallabies (*Macropus eugenii*) respond to visual but not acoustic cues from predators. *Behavioral Ecology*, **11**: 528–535.

Blumstein, D. T., Mari, M., Daniel, J. C., Ardron, J. G., Griffin, A. S. y Evans, C. S. 2002. Olfactory predator recognition: wallabies may have to learn to be wary. *Animal Conservation*, **5**: 87–93.

Blumstein, D. T., Ozgul, A., Yovovich, V., Van Vuren, D. H. y Armitage, K. B. 2006. Effect of predation risk on the presence and persistence of yellow–bellied marmot (*Marmota flaviventris*) colonies. *Journal of Zoology*, **270**: 132–138.

Boag, B. y Mlotkiewicz, J. A. 1994. Effect of odor derived from lion faeces on behavior of wild rabbits. *Journal of Chemical Ecology*, **20**: 631–637.

Boissy, A. 1995. Fear and fearfulness in animals. *The Quarterly Review of Biology*, **70**: 165–191.

Boriss, H., Boersma, M. y Wiltshire, K. H. 1999. Trimethylamine induces migration of waterfleas. *Nature*, **398**: 382.

Borowski, Z. 2002. Individual and seasonal differences in antipredatory behaviour of root voles - a field experiment. *Canadian Journal of Zoology*, **80**: 1520–1525.

Brodin, R., Mikolajewski, D.J. y Johansson F. 2006. Behavioural and life history effects of predator diet cues during ontogeny in damselfly larvae. *Oecologia*, **148**: 162–169.

Brown, C. y Warburton, K. 1997. Predator recognition and anti–predator responses in the rainbowfish *Melanotaenia eachamensis*. *Behavioral Ecology and Sociobiology*, **41**: 61–68.

Brown, G. E. y Zachar, M. M. 2002. Chemical predator inspection in a characin fish (*Hemigrammus erythrozonus*, Characidae, Ostariophysi): the effects of mixed predator diets. *Ethology*, **108**: 451–461.

Brown, G. E., Paige, J. A. y Godin, J–G. J. 2000. Chemically mediated predator inspection behaviour in the absence of predator visual cues by a characin fish *Animal Behaviour*, **60**: 315–321.

Brown, G. E., Poirier, J. F. y Adrian, J. C. Jr, 2004. Assessment of local predation risk: the role of subthreshold concentrations of chemical alarm cues. *Behavioral Ecology*, **15**: 810–815.

Buchanan, K. L. 2000. Stress and the evolution of condition–dependent signals. *Trends in Ecology and Evolution*, **15**: 156–160.

Burwash, M. D., Tobin, M. E., Woolhouse, A. D. y Sullivan, T. P. 1998 a. Laboratory evaluation of predator odors for eliciting an avoidance response in roof rats (*Rattus rattus*). *Journal of Chemical Ecology*, **24**: 49–66.

Burwash, M. D., Tobin, M. E., Woolhouse, A. D. y Sullivan, T. P. 1998 b. Field testing synthetic predator odors for roof rats (*Rattus rattus*) in Hawaiian Macadamia nut orchards. *Journal of Chemical Ecology*, **24**: 603–630.

Butler, J. M. y Roper, T. J. 1994. Escape tactics and alarm responses in badgers *Meles meles*: a field experiment. *Ethology*, **99**: 313–322.

Caine, N. G. y Weldon, P. J. 1989. Responses by red–bellied tamarins (*Saguinus labiatus*) to fecal scents of predatory and non-predatory neotropical mammals. *Biotropica*, **21**: 186–189.

Calder, C. J. y Gorman, M. L. 1991. The effects of red fox *Vulpes vulpes* faecal odours on the feeding behaviour of Orkney voles *Microtus arvalis*. *Journal of Zoology,* London, **224**: 599–606.

Cameron, E. Z. y du Toit, J. T. 2005. Social influences on vigilance behaviour in giraffes, *Giraffa camelopardalis*. *Animal Behaviour*, **69**: 1337–1344.

Cannicci, S., Morino, L. y Vannini, M. 2002. Behavioural evidence for visual recognition of predators by the mangrove climbing crab *Sesarma leptosoma*. *Animal Behaviour*, **63**: 77–83.

Caro, T. 2005. *Antipredator defenses in birds and mammals*. Chicago: The University of Chicago Press.

Childress, M. J. y Lung, M. A. 2003. Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour*, **66**: 389–398.

Chivers, D. P., Mirza, R. S., Bryer, P. J. y Kiesecker, J. M. 2001. Threat-sensitive predator avoidance by slimy sculpins: understanding the importance of visual versus chemical information. *Canadian Journal of Zoology*, **79**: 867–873.

Cowlishaw, G. 1997. Trade-offs between foraging and predation risk determine habitat use in a desert baboon population. *Animal Behaviour*, **53**: 667–686.

Cox, J. G. y Lima, S. L. 2006. Naiveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. *Trends in Ecology and Evolution*, **21**: 674–680.

Creel, S. 2001. Social dominance and stress hormones. *Trends in Ecology and Evolution*, **16**: 491–497

Creel, S., Winnie, J. Jr, Maxwell, B., Hamlin, K. y Creel, M. 2005. Elk alter habitat selection as an antipredator response to wolves. *Ecology*, **86**: 3387–3397.

Cresswell, W., Lind, J., Kaby, U., Quinn, J. L. y Jakobsson, S. 2003. Does an opportunistic predator preferentially attack nonvigilant prey? *Animal Behaviour*, **66**: 643–648.

Curio, E. 1993: Proximate and developmental aspects of antipredator behaviour. *Advances in the Study of Behavior*, **22**: 135–238.

Daly, J. W., Myers, C. W., Warnick, J. E. y Albuquerque E. X. 1980. Levels of batrachotoxin and lack of sensitivity to its action in dart poison fogs (*Phyllobates*). *Science*, **208**: 1383–1385.

Daly, M., Wilson, M., Behrends, P. R. y Jacobs, L. F. 1990. Characteristics of kangaroo rats, *Dipodomys merriami*, associated with differential predation risk. *Animal Behaviour*, **40**: 380–389.

Delibes, M., e Hiraldo, F. 1981. The rabbit as prey in the Iberian mediterranean ecosystem. *En*: Myers, K. y MacInnes, C. D. (Eds.). *Proceedings of the World Lagomorph Conference*, pp. 614–622. Ontario: University of Guelph.

DeVries, A. C., Glasper, E. R. y Detillion, C. E. 2003: Social modulation of stress responses. *Physiology and Behavior*, **79**: 399–407.

Di Blanco, Y. y Hirsch, B. T. 2006 Determinants of vigilance behavior in the ring-tailed coati (*Nasua nasua*): the importance of within-group spatial position.*Behavioral Ecology and Sociobiology*, **61**: 173–182.

Dial, B. E. y Schwenk, K. 1996. Olfaction and predator detection in *Coleonys brevis* (Squamata, Eublepharidae), with comments on the functional significance of buccal pulsing in geckos. *Journal of Experimental Zoology*, **276**: 415–424.

Dickman, C. R. 1992. Predation and habitat shift in the house mouse, *Mus domesticus*. *Ecology*, **73**: 313–322.

Dumbacher, J. P., Beehler, B. M., Spande, T. F., Garraffo, H. M. y Daly, J. W. 1992. Homobatrachotoxin in the genus Pitohui: Chemical defense in birds? *Science*, **258**: 799–801.

Durant, S. M. 2000. Predator avoidance, breeding experience and reproductive success in endangered cheetahs, *Acinonyx jubatus*. *Animal Behaviour*, **60**: 121–130.

Ebensperger, L.A., Hurtado, M.J. y Ramos-Jiliberto, R. 2006. Vigilance and collective detection of predators in degus (*Octodon degus*). *Ethology*, **112**: 879–887.

Eilam, D., Dayan, T., Ben–Heliyahu, S., Schulman, I., Shefer, G. y Hendrie, C. A. 1999. Differential behavioural and hormonal responses of voles and spiny mice to owl calls. *Animal Behaviour*, **58**: 1085–1093.

Elgar, M. A. 1989. Predator vigilance and group size in mammals and birds: A critical review of the empirical vigilance. *Biological Reviews*, **64**: 13–33.

Endler, J. A. 1978. A predator's view of animal color pattern. *Evolutionary Biology*, **11**: 319–364.

Endler, J. A. 1991. Interaction between predators and prey. *En*: Krebs, J. R. y Davies, N. B. (Eds.). *Behavioural Ecology: An Evolutionary Approach*, 3rd edn., pp. 169–196. Oxford: Blackwell Science.

Endres, T., Apfelbach, R. y Fendt, M. 2005. Behavioral changes induced in rats by exposure to trimethylthiazoline, a component of fox odor. *Behavioral Neuroscience*, **119**: 1004–1010.

Epple, G., Mason, J. R., Nolte, D. L y Campbell, D. L. 1993. Effects of predator odors on feeding in the mountain beaver (*Aplodontia rufa*). *Journal of Mammalogy*, **74**: 715–722.

Fairbanks, B. y Dobson, F. S. 2007. Mechanisms of the group–size effect on vigilance in Columbian ground squirrels: dilution versus detection. *Animal Behaviour*, **73**: 115–123.

Farr J. A. 1975. The role of predation in the evolution of social behavior in natural populations of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Evolution*, **29**: 151–158.

Fendt, M. 2006. Exposure to urine of canids and felids, but not of herbivores, induces defensive behavior in laboratory rats. *Journal of Chemical Ecology*, **32**: 2617–2627.

Fendt, M., Endres, T. y Apfelbach, R. 2003. Temporary inactivation of the bed nucleus of the stria terminalis but not of the amygdala blocks freezing induced by trimethylthiazoline, a component of fox feces. *Journal of Neuroscience*, **23**: 23–28.

Fenn, M. G. P. y Macdonald, D. W. 1995. Use of middens by red foxes: risk reverses rhythms of rats. *Journal of Mammalogy*, **76**: 130–136.

Fernández-Juricic E. y Kacelnik, A. 2004. Information transfer and gain in flocks: the effects of quality and quantity of social information at different neighbour distances. *Behavioral Ecology and Sociobiology*, **55**: 502–511.

Ferrari, M. C. O. y Chivers, D. P. 2006. Learning threat–sensitive predator avoidance: how do fathead minnows incorporate conflicting information? *Animal Behaviour*, **71**: 19–26.

Ferrari, M. C. O., Messier, F. y Chivers, D. P. 2006. The nose knows: minnows determine predator proximity and density through detection of predator odours. *Animal Behaviour*, **72**: 927–932.

FitzGibbon, C. D. 1989. A cost to individuals with reduced vigilance in groups of Thomson's gazelles hunted by cheetahs. *Animal Behaviour*, **37**: 508–510.

FitzGibbon, C. D. 1990. Why do hunting cheetahs prefer male gazelles? *Animal Behaviour*, **40**: 837–845

Flier, J., Edwards, M. W., Daly, J. W. y Myers, C. W. 1980. Widespread occurrence in frogs and toads of skin compounds interacting with the ouabain site of Na+–K+–ATPase. *Science*, **208**: 503–505.

Flowers, M. A. y Graves, B. M. 1997 Juvenile toads avoid chemical cues from snake predators *Animal Behaviour*, **53**: 641–646.

Fluck, E., Hogg, S., Mabbutt, P. S. y File, S. E. 1996. Behavioural and neurochemical responses of male and female chicks to cat odour. *Pharmacology, Biochemistry and Behavior*, **54**: 85–91.

Foam, P. E., Harvey, M. C., Mirza, R. S. y Brown, G. E. 2005. Heads up: juvenile convict cichlids switch to threat–sensitive foraging tactics based on chemosensory information. *Animal Behaviour*, **70**: 601–607.

Forsgren, E. y Magnhagen, C. 1993. Conflicting demands in sand gobies: predators influence reproductive behaviour. *Behaviour*, **126**: 125–135.

Francis, A. M., Hailman, J. P. y Woolfende, G. E. 1989. Mobbing by Florida scrub jays: behaviour, sexual asymmetry, role of helpers and ontogeny. *Animal Behaviour*, **38**: 795–816.

Glück, E. 1987. An experimental study of feeding, vigilance and predator avoidance in a single bird. *Oecologia*, **71**, 268–272.

Gorman, M. L. 1984. The response of prey to stoat (*Mustela erminea*) scent. *Journal of Zoology,* London, **202**: 419–423.

Gosling S. D. 2001. From mice to men: what can we learn about personality from animal research? *Psychological Bulletin*, **127**: 45–86.

Graves B. M., Summers C., Olmstead K. L. 1993. Sensory mediation of aggregation among postmetamorphic *Bufo cognatus*. *Journal of Herpetology*, **27**: 315–319.

Greene, H. W. y McDiarmid, R. W. 1981. Coral snake mimicry: Does it occur? *Science*, **213**: 1207–1212.

Griffin, A. S., Blumstein, D. T. y Evans, C. S. 2000. Training captive–bred or translocated animals to avoid predators. *Conservation Biology*, **14**: 1317–1326.

Grostal, P. y Dicke, M. 1999. Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. *Behavioral Ecology*, **10**: 422–427.

Grostal, P. y Dicke, M. 2000. Recognising one's enemies: a functional approach to risk assessment by prey. *Behavioral Ecology and Sociobiology*, **47**: 258–264.

Gustafson, E. J. y VanDruff, L. W. 1990. Behavior of black and gray morphs of Sciurus carolinensis in an urban environment. *American Midland Naturalist*, **123**: 186–192.

Hamilton, W. D. 1971. The geometry of the selfish herd. *Journal of Theoretical Biology*, **31**: 295–311.

Hansell, M. 2000. *Bird nests and construction behaviour*. Cambridge: Cambridge University Press.

Hartman, E. J. y Abrahams, M. V. 2000. Sensory compensation and the detection of predators: the interaction between chemical and visual information. *Proceedings of the Royal Society B, London*, **267**: 571–575.

Hass, C. C. y Valenzuela, D. 2002. Anti–predator benefits of group living in white–nosed coatis (*Nasua narica*). *Behavioral Ecology and Sociobiology*, **51**: 570–578.

Hebert, P. D. N. y Grewe, P. M. 1985. *Chaoborus*–induced shifts in the morphology of *Daphnia ambigua*. *Limnology and Oceanography*, **30**: 1291–1297.

Heikkilä, J., Kaarsalo, K., Mustonen, O. y Pekkarinen, P. 1993. Influence of predation risk on early development and maturation in three species of *Clethrionomys* voles. *Annales Zoologici Fennici*, **30**: 153–161.

Heithaus, M. R. y Dill, L. M. 2002. Food availability and tiger shark predation risk influence bottlenose dolphin habitat use. *Ecology*, **83**: 480–491.

Helfman, G. S. 1989. Threat–sensitive predator avoidance in damselfish–trumpetfish interactions. *Behavioral Ecology and Sociobiology*, **24**: 47–58.

Heth, G., Beilers, A. y Nevo, E. 1988. Adaptive variation of pelage color within and between species of the subterranean mole rat (*Spalax ehrenbergi*) in Israel. Oecologia, 74: 617–622.

Hews, D. K. 1988. Alarm response in larval western toads, *Bufo boreas*: release of larval chemicals by a natural predator and its effect on predator capture efficiency. *Animal Behaviour*, **36**: 125–133.

Hölker, F. y Stief, P. 2005 Adaptive behaviour of chironomid larvae (*Chironomus riparius*) in response to chemical stimuli from predators and resource density. *Behavioral Ecology and Sociobiology*, **58**: 256–263.

Horat, P. y Semlitsch, R. D. 1994. Effects of predation risk and hunger on the behaviour of two species of tadpoles. *Behavioral Ecology and Sociobiology*, **34**: 393–401.

Hubbard, D. T., Blanchard, D. V., Yan, M., Markham, C. M. Gervacio, A., Chun–I, L. y Blanchard, R. J. 2004. Development of defensive behaviour and conditioning to cat odor in the rat. *Physiology and Behavior*, **80**: 525–530.

Inglis, I. R. 1979: Visual bird scarers an ethological approach. *En*: Wright, E. N., Inglis, I. R. y Feare, C. J. (Eds.). *Bird Problems in Agriculture*, pp. 121—143. London: MAFF Publishers.

Inglis, I. R. y Shepherd, D. S. 1990. Communication. En: Monaghan, P. y Wood–Gush, D. (Eds.). *Managing the behaviour of animals*. London: Chapman y Hall.

Ireland, D. H., Wirsing, A. J. y Murray, D. L. 2007. Phenotypically plastic responses of green frog embryos to conflicting predation risk. *Oecologia*, **153**: 162–168.

Jaksic, F. M. y Soriguer, R. C. 1981. Predation upon the European rabbit (*Oryctolagus cuniculus*) in Mediterranean habitats of Chile and Spain: a comparative analysis. *Journal of Animal Ecology*, **50**: 269–281.

Jedrzejewski, W. y Jedrzejewska, B. 1990. Effect of a predator's visit on the spatial distribution of bank voles: experiments with weasels. *Canadian Journal of Zoology*, **68**: 660–666.

Jedrzejewski, W., Rychlik, L. y Jedrzejewska, B. 1993. Responses of bank voles to odours of seven species of predators: experimental data and their relevance to natural predator–vole relationships. *Oikos*, 68: 251–257.

Jennions, M. D., Backwell, P. R. Y., Murai, M. y Christy, J. H. 2003. Hiding behaviour in fiddler crabs: how long should prey hide in response to a potential predator? *Animal Behaviour*, **66**: 251–257.

Johnson, R. P. 1973. Scent marking in mammals. *Animal Behaviour*, **21**: 521–535.

Jones, R. B y Roper, T. J. 1997. Olfaction in the domestic fowl: a critical review. *Physiology and Behavior*, **62**: 1009–1018.

Kats, L. B. y Dill, L. M. 1998. The scent of death: chemosensory assessment of predation risk by prey animal. *Ecoscience*, **5**: 361–394.

Kavaliers, M. y Choleris, E. 2001. Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neuroscience and Biobehavioral Reviews*, **25**: 577–586.

Kelley, J. L., Evans, J. P., Ramnarine, I. W. y Magurran, A. E. 2003. Back to school: can antipredator behaviour in guppies be enhanced through social learning? *Animal Behaviour*, **65**: 655–662.

Kesavaraju, B., Damal, K. y Juliano, S. A. 2007. Threat-sensitive behavioral responses to concentrations of water-borne cues from predation. *Ethology*, **113**: 199–206.

Kiesecker, J. M., Chivers, D. P. y Blaustein, A. R. 1996. The use of chemical cues in predator recognition by western toad tadpoles. *Animal Behaviour*, **52**: 1237–1245.

Kiltie, R. A. 1989. Countershading: Universally deceptive or deceptively universal. *Trends in Ecology and Evolution*, **3**: 21–23.

King, G. M. 1984. Metabolism of trimethylamine, choline and glycine betaine by sulfate–reducing and methanogenic bacteria in marine sediments. *Applied and Environmental Microbiology*, **48**: 719–725.

Kleiman, D. 1966. Scent marking in the Canidae. S*ymposium of the Zoological Society of London*, **18**: 167–177.

Korpimäki, E., Norrdahl, K. y Valkama, J. 1994. Reproductive investment under fluctuating predation risk – microtine rodents and small mustelids. *Evolutionary Ecology*, **4**: 357–368.

Koskela, E. e Ylönen, H. 1995. Suppressed breeding in the field vole (*Microtus agrestis*): an adaptation to cyclically fluctuating predation risk. *Behavioral Ecology*, **6**: 311–315.

Kotler, B. P., Brown, J. S., Dall, S. R. X., Gresser, S., Ganey, D. y Bouskila A. 2002. Foraging games between gerbils and their predators: temporal dynamics of resource depletion and apprehension in gerbils. *Evolutionary Ecology Research*, **4**: 495–518.

Kusch, R. C., Mirza, R. S. y Chivers, D. P. 2004. Making sense of predator scents: investigating the sophistication of predator assessment abilities of fathead minnows. *Behavioral Ecology and Sociobiology*, **55**: 551–555.

Landys, M. M., Ramenofsky, M. y Wingfield, J. C. 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress–related levels in the regulation of periodic life processes. *General and Comparative Endocrinology*, **148**: 132–149.

Lariviere, S. y Messier, F. 1996. Aposematic behaviour in the striped skunk, *Mephitis mephitis*. *Ethology*, **102**: 986–992.

Laurila, A. 2000. Behavioural responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. *Oikos*, **88**: 159–168.

Licht, T. 1989. Discriminating between hungry and satiated predators: the response of guppies (*Poecilia reticulata*) from high and low predation sites. *Ethology*, **82**: 238–243.

Lima, S. L. y Dill, L. M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**: 619–640.

Lima, S. L. 1998 a. Nonlethal effects in the ecology of predator–prey interactions. What are the ecological effects of anti–predator decision–making? *BioScience*, **48**: 25–34.

Lima, S. L. 1998 b. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive and ecological perspectives. *Advances in the Study of Behavior*, **27**: 215–290.

Lingle S. y Pellis S. M. 2002. Fight or flight? Antipredator behavior and the escalation of coyote encounters with deer. *Oecologia*, **131**: 154–164.

Loose, C. J. y Dawidowicz, P. 1994. Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. *Ecology*, **75**: 2255–2263.

Loughry, W. J. 1988. Population differences in how black–tailed prairie dogs deal with snakes. *Behavioral Ecology and Sociobiology*, **22**: 61–67.

Loughry, W. J. 1993. Determinants of time allocation by adult and yearling black–tailed prairie dogs. *Behaviour*, **124**: 23–43.

Luttbeg, B. y Kerby, J. L. 2005. Are scared prey as good as dead? T*rends in Ecology and Evolution*, **20**: 416–418.

Lutterschmidt, W. I., Marvin, G. A. y Hutchison, V. H. 1994. Alarm response by a plethodontid salamander (*Desmognathus ochrophaeus*): conspecific and heterospecific 'Schreckstoff'. *Journal of Chemical Ecology*, **20**: 2751–2757.

Macdonald, D. W. 1977. On food preference in red fox. *Mammal Review*, **7**: 7–23.

Magalhães, S., Tudorache, C., Montserrat, M., van Maanen, R., Sabelis, M. W. y Janssen, A. 2005. Diet of intraguild predators affects antipredator behavior in intraguild prey. *Behavioral Ecology*, **16**: 364–370.

Magnhagen, C. 1991. Predation risk as a cost of reproduction. *Trends in Ecology and Evolution*, **6**: 183–186.

Magurran A. E., Irving, P. W. y Henderson, P. A. 1996. Is there a fish alarm pheromone? A wild study and critique. *Proceedings of the Royal Society, B*, **263**: 1551–1556.

Martín, J. y López, P. 1999. When to come out from a refuge: risk-sensitive and state-dependent decisions in an alpine lizard. *Behavioral Ecology*, **10**: 487–492.

Martín, J. y López, P. 2001. Are fleeing "noisy" lizards signalling to predators? *Acta Ethologica*, **3**: 95–100.

Mason, J. R., Clark, L. y Shaw, P. S. 1991. Ortho–aminoacetophenone repellency to birds: Similarities to methyl anthranilate. *Journal of Wildlife Management*, **55**: 334–340.

Mathis, A. y Smith, R. F. J. 1993. Intraspecific and cross–superorder responses to chemical alarm signals by brook stickleback. *Ecology*, **74**: 2395–2404.

Matteri, R. L., Carroll, J. A. y Dyer, C. J. 2001. Neuroendocrine responses to stress. *En*: Moberg, G. P. y Mench, J.A. (Eds). *The biology of animal stress. Basic principles and implications for animal welfare*, pp 43–76. Oxon: CABI Publishing.

McAdam, A. G. y Kramer, D. L. 1998. Vigilance as a benefit of intermittent locomotion in small mammals. *Animal Behaviour*, **55**: 109–117.

McDonough C. M. y Loughry W. J. 1995. Influences on vigilance in nine-banded armadillos. *Ethology*, **100**: 50–60.

Milinski, M. 1977. Experiments on the selection by predators against spatial oddity of their prey. *Zeitschrift für Tierpsychologie*, **43**: 311–325.

Moreno S., Villafuerte R. y Delibes M. 1996. Cover is safe during the day but dangerous at night: the use of vegetation by European wild rabbits. *Canadian Journal of Zoology*, **74**: 1656–1660.

Möstl, E. y Palme, R. 2002. Hormones as indicators of stress. *Domestic Animal Endocrinology*, **23**: 67–74.

Murray, D. L. 2002. Differential body condition and vulnerability to predation in snowshoe hares. *Journal of Animal Ecology*, **71**: 614–625.

Murray, D. L. y Jenkins, C. L. 1999. Perceived predation risk as a function of predator dietary cues in terrestrial salamanders. *Animal Behaviour*, **57**: 33–39.

Mykytowycz, R. 1968. Territorial marking by rabbits. *Scientific American*, **218**: 116–126.

Mykytowycz, R. y Gambale, S. 1969. The distribution of dung–hills and the behaviour of freeliving wild rabbits, *Oryctolagus cuniculus* (L.), on them. *Forma et functio,* **1**: 333–349.

Nilsson, P. A., Brönmark, C. y Pettersson, L. B. 1995. Benefits of a predtor-induced morphology in crucian carp. *Oecologia*, **104**: 291–296.

Nolte, D. L., Mason, J. R., Epple, G., Aronov, E. y Campbell D. L. 1994. Why are predator urines aversive to prey? *Journal of Chemical Ecology*, **20**: 1505–1516.

Norrdahl, K. y Körpimaki, E. 1998. Does mobility or sex of voles affect risk of predation by mammalian predators? *Ecology*, **79**: 226–232.

Norton, S. F. 1988. Role of the gastropod shell and operculum in inhibiting predation by fishes. *Science*, **241**: 92–94.

Orrock, J. L., Danielson, B. J. y Brinkerhoff, R. J. 2004. Rodent foraging is affected by indirect, but not by direct, cues of predation risk. *Behavioral Ecology*, **15**: 433–437.

Orrock, J. L., Danielson, B. J. 2004. Rodents balancing a variety of risks: invasive fire ants and indirect and direct indicators of predation risk. *Oecologia*, **140**: 662–667

Parer, I. 1977. The population ecology of the wild rabbit, *Oryctolagus cuniculus* (L.) in a mediterranean type climate in New South Wales. *Australian Wildlife Research*, **4**: 171–205.

Pavey, C. R. y Smyth, A. K. 1998. Effects of avian mobbing on roost use and diet of powerful owls, *Ninox strenua*. *Animal Behaviour*, **55**: 313–318.

Petterson, L. B., Nilsson, P. A. y Brönmark, C. 2000. Predator recognition and defence strategies in crucian carp, *Carassius carassius*. *Oikos*, **88**: 200–212.

Pfeiffer, W., Riegelbauer, G., Meier, G. y Scheibler, B. 1985. Effects of hypoxanthine–3(N)–oxide and hypoxanthine–1(N)–oxide on central nervous excitation of the black tetra *Gymnocorymbus ternetzi* (Characidae, Ostariophysi, Pisces) indicated by dorsal light response. *Journal of Chemical Ecology*, **11**: 507–524.

Powell, F. y Banks, P. B. 2004. Do house mice modify their foraging behaviour in response to predator odours and habitat? *Animal Behaviour*, **67**: 753–759.

Pravosudov, V. V. y Grubb, T. C. 1999. Effects of dominante on vigilance in avian social groups. *The Auk,* **116**: 241–246.

Preisser, E. L., Bolnick, D. I. y Benard M. F. 2005. Scared to death? The effects of intimidation in predator–prey interactions. *Ecology*, **86**: 501–509.

Pusenius, J. y Ostfeld R. S. 2000. Effects of stoat's presence and auditory cues indicating its presence on tree seedling predation by meadow voles. *Oikos*, **91**: 123–130.

Puttlitz, M. H., Chivers, D. P., Kiesecker, J. M. y Blaustein, A. R. 1999. Threat-sensitive predator avoidance by larval Pacific treefrogs (Amphibia, Hylidae). *Ethology*, **105**: 449–456.

Ralls, K. 1971. Mammalian scent marking. *Science*, **171**: 443–449.

Relyea, R. A. 2007. Getting out alive: how predators affect the decision to metamorphose. *Oecologia*, **152**: 389–400.

Robinette, R. L. y Ha, J. C. 2001. Social and ecological factors influencing vigilante by northwestern crows, *Corvus caurinus*. *Animal Behaviour*, **62**: 447–452.

Robinson, S. R. 1980. Antipredator behaviour and predator recognition in Belding's ground squirrels. *Animal Behaviour*, **28**: 840–852.

Rödl, T., Berger, S., Romero, L. M. y Wikelski, M. 2007. Tameness and stress physiology in a predator–naive island species confronted with novel predation threat. *Proceedings of the Royal Society, B.*, **274**: 577–582.

Rohr, J. R. y Madison, D. M. 2001. A chemically mediated trade–off between predation risk and mate search in newts. *Animal Behaviour*, **62**: 863–869.

Sage, B. L. 1962. Albinism and melanism in birds. *Breeding Birds*, **55**: 201–225.

Sapolsky, R. M. 1992. Neuroendocrinology of the stress–response. *En*: Becker, J. B., Breedlove, S. M. y Crews, D. (Eds.). *Behavioural Endocrinology*, pp. 287–324. Cambridge, Massachusetts: MIT Press.

Scannell, J., Roberts, G. y Lazarus, J. 2001. Prey scan at random to evade observant predators. *Proceedings of the Royal Society of London. Serie B*, **268**: 541–547.

Scheuerlein, A., van't Hof, T. J. y Gwinner, E. 2001. Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). *Proceedings of the Royal Society of London, B*, **268**: 1575–1582.

Schradin, C. 2000. Confusion effect in a reptilian and a primate predator *Ethology*, **106**: 691–700.

Sih, A., Bell, A. M., Johnson, J. C., Ziemba, R. E. 2004. Behavioral syndromes: an integrative overview. *The Quarterly Review of Biology*, **79**: 241–79.

Slotow, R. y Rothstein, S. I. 1995. Influence of social status, distance from cover, and group size on feeding and vigilance in white–crowned sparrows. *The Auk*, **112**: 1024–1031.

Smith, M. E. y Belk, M. C. 2001. Risk assessment in western mosquitofish (*Gambusia affinis*): do multiple cues have additive effects? *Behavioral Ecology and Sociobiology*, **51**: 101–107.

Sneddon, I. A. 1991. Latrine use by the European rabbit (*Oryctolagus cuniculus*). *Journal of Mammalogy*, **72**: 769–775.

Stanford, C. B. 2002. Avoiding predators: Expectations and evidence in primate antipredator behaviour. *International Journal of Primatology*, **23**: 741–757.

Stapley, J. 2004. Do mountain log skinks (*Pseudemoia entrecasteauxii*) modify their behaviour in the presence of two predators? *Behavioral Ecology and Sociobiology*, **56**: 185–189.

Stenhouse, I. J., Gilchrist, H. G., Montevecchi, W. A. 2005. An experimental study examining the anti–predator behaviour of Sabine's gulls (*Xema sabini*) during breeding. *Journal of Ethology*, **23**: 103–108.

Stoddart, D. M. 1976. Effect of the odor of weasels (*Mustela nivalis* L.) on trapped samples of their prey. *Oecologia*, **22**: 439–441.

Stoddart, M. 1982. Does trap odour influence estimation of population size of the short–tailed vole, *Microtus agrestis*? *Journal of Animal Ecology*, **51**: 375–386.

Stoner, C. J., Caro, T. M. y Graham, C. M. 2003. The adaptive significance of coloration in lagomorphs. *Biological Journal of the Linnean Society*, **79**: 309–328.

Sullivan, A. M., Maerz, J. C. y Madison, D. M. 2002. Anti–predator response of red–backed salamanders (*Plethodon cinereus*) to chemical cues from garter snakes (*Thamnophis sirtalis*): laboratory and field experiments. *Behavioral Ecology and Sociobiology*, **51**: 227–233.

Sullivan, T. P., Crump, D. R. y Sullivan, D. S. 1988. Use of predator odors as repellents to reduce feeding damage by herbivores. III. Montane and meadow voles (*Microtus montanus* and *Microtus pennsylvanicus*). *Journal of Chemical Ecology*, **14**: 363–377.

Sundell, J e Ylönen, H. 2004. Behaviour ad choice of refuge by voles under predation risk. *Behavioral Ecology and Sociobiology*, **56**: 263–269.

Swaisgood, R. R., Owings, D. H. y Rowe, M. P. 1999. Conflict and assessment in a predator–prey system: ground squirrels versus rattlesnakes. *Animal Behaviour*, **57**: 1033–1044.

Symula, R., Schulte, R. y Summers, K. 2001. Molecular phylogenetic evidence for a mimetic radiation in Peruvian poison frogs supports a Mullerian mimicry hypothesis. *Proceedings of the Royal Society*, B, **268**: 2415–2421.

Tchabovsky, A. V., Popov, S. V. y Krasnov, B. R. 2001. Intra- and interspecific variation in vigilance and foraging of two gerbillid rodents, *Rhombomys opimus* and *Psammomys obesus*: the effect of social environment. *Animal Behaviour*, **62**: 965–972.

Teplitsky, C., Plénet, S. y Joly, P. 2005. Costs and limits of dosage response to predation risk: to what extent can tadpoles invest in anti–predator morphology? *Oecologia*, **145**: 364–370.

Tobin, M. E., Engeman, R. M. y Sugihara, R. T. 1995. Effects of mongoose odors on rat capture success. *Journal of Chemical Ecology*, **21**: 635–639.

Topál, J. y Csányi, V. 1994. The effect of eye–liked schema on shuttling activity of wild house mice (*Mus musculus domesticus*): context dependent threatening aspects of the eyespot patterns. *Animal Learning & Behaviour*, **22**: 96–102.

Touma, C. y Palme, R. (2005) Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of a validation. *Annals of the New York Academy of Science*, **1046**: 54–74

Turner, A. M., Turner, S. E. y Lappi, H. M. 2006. Learning, memory and predator avoidance by freshwater snails: effects of experience on predator recognition and defensive strategy. *Animal Behaviour*, **72**: 1443–1450.

van der Veen I. T. 2002. Seeing is believing: information about predators influences yellowhammer behavior. *Behavioral Ecology and Sociobiology*, **51**: 466–471.

Vernet–Maury, E. 1980. Trimethyl–thiazoline in fox feces: a natural alarming substance for the rat, p. 407. *En*: van der Starre, H. (ed.). *Proceedings of the VII International Symposium on Olfaction and Taste VII*. London: IRL Press.

Vernet–Maury, E., Polak, E. H. y Demael, A. 1984. Structure–activity relationship of stress-inducing odorants in the rat. *Journal of Chemical Ecology*, **10**: 1007–1018.

Vitale, A. F. 1989. Changes in anti–predator responses of wild rabbits, *Oryctolagus cuniculus* (L.), with age and experience. *Behaviour*, **110**: 47–61.

von Frisch, K. 1941. Über einen Schreckstoff der Fischhaut und seine biologische Bedeutung. *Zeitschrift für vergleichende Physiologie*, **29**: 46–145.

von Holst, D. 1998. The concept of stress and its relevance for animal behavior. *Advances in the Study of Behavior*, **27**: 1–131.

White, K. S. y Berger, J. 2001 Antipredator strategies of Alaskan moose: are maternal trade–offs influenced by offspring activity? *Canadian Journal of Zoology*, **79**: 2055–2062.

Winnie, J. y Creel S. (2007). Sex–specific behavioural responses of elk to spatial and temporal variation in the threat of wolf predation. *Animal Behaviour*, **73**: 215–225.

Wit, C. A. de 1982. Resistance of the prairie vole (*Microtus ochrogaster*) and the woodrat (*Neotoma floridana*), in Kansas, to venom of the Osage copperhead (*Agkistrodon contortrix phaeogaster*). *Toxicon*, **20**: 709–714.

Wit, C. A. de y Westrom, B. R. 1987. Venom resistance in the hedgehog, *Erinaceus europaeus*: Purification and identification of macroglobulin inhibitors as plasma antihemorragic factors. *Toxicon*, **25**: 315–323.

Wolff, J. O. 2003. Laboratory studies with rodents: facts or artefacts? *BioScience*, 53, 421–427.

Wood, W. F., Sollers, B. G., Dragoo, G. A. y Dragoo, J. W. 2002. Volatile components in defensive spray of the hooded skunk, *Mephitis macoura*. *Journal of Chemical Ecology*, **28**: 1865–1870.

Woodley, C. M. y Peterson, M. S. 2003. Measuring responses to simulated predation threat using behavioral and physiological metrics: the role of aquatic vegetation. *Oecologia*, **136**: 155–160.

Ydenberg, R. C. y Dill, L. M. 1986. The economics of fleeing from predators. *Advances in the study of Behavior*, **16**: 229–249.

Ylönen, H. y Ronkainen, H. 1994. Breeding suppression in the bank vole as antipredatory adaptation in a predictable environment. *Evolutionary Ecology*, **8**: 658–666.

Zhang, J. X., Sun, L. y Novotny, M. 2007. Mice respond differently to urine and its major volatile constituents from male and female ferrets. *Journal of Chemical Ecology*, **33**: 603–612.

Zhao, X., Ferrari, M. C. O. y Chivers, D. P. 2006. Threat-sensitive learning of predator odours by a prey fish. *Behaviour*, **143**: 1103–1121.

CAPÍTULO 2

Las respuestas comportamentales y fisiológicas de los conejos al olor de un depredador

Manuscrito original del artículo publicado en Animal Behaviour:

Monclús, R., Rödel, H. G., von Holst, D. & Miguel, F. J. 2005. Behavioural and physiological responses of naïve European rabbits to predator odour. Animal Behaviour, 70: 753–761.

Las respuestas comportamentales y fisiológicas de los conejos al olor de un depredador

Resumen

Los animales desarrollan diversas estrategias antidepredatorias en respuesta a la presencia de señales químicas de los mamíferos depredadores. Sin embargo, no existe un acuerdo general en relación a si el reconocimiento de los olores de los depredadores depende de la experiencia. Llevamos a cabo un experimento con conejos *Oryctolagus cuniculus* sin experiencia previa con depredadores e investigamos (1) si poseían un mecanismo para el reconocimiento del olor de los depredadores y (2) cómo respondían tanto comportamental como fisiológicamente a ese olor. Utilizamos heces de zorro *Vulpes vulpes* como fuente del olor del depredador y heces de oveja *Ovis aries* como olor control de un no depredador. Los experimentos se llevaron a cabo en pequeñas instalaciones exteriores donde los animales se alojaron en solitario. Registramos los patrones de vigilancia, de actividad y de alimentación, y los cambios en glucocorticoides y masa corporal. Los conejos mostraron una respuesta antidepredatoria clara a la presencia de heces de zorro, mientras que se comportaron de una manera neutral en respuesta al olor de oveja. La respuesta consistió en un aumento de la evitación y de la vigilancia mientras comían, y un aumento de la investigación antes de comer. Además, los conejos mostraron una respuesta fisiológica de alarma, es decir, una mayor capacidad de la respuesta del sistema adrenocortical y pérdida de peso. Sin embargo, el presupuesto total de actividad, medido como el tiempo que pasaban fuera de la madriguera, el tiempo empleado en comer, y la cantidad de comida ingerida se mantuvieron muy estables a lo largo del experimento. Concluimos que los conejos recocieron los olores de los depredadores y que este reconocimiento fue independiente de la experiencia.

Behavioural and physiological responses of naïve European rabbits to predator odour

Abstract

Animals display a variety of antipredator strategies in response to the presence of chemical cues from mammalian predators. Nevertheless, there is no general agreement as to whether recognition of predator odours is dependent upon experience. We conducted an experiment on European rabbits *Oryctolagus cuniculus* naïve to any contact with predators and we investigated (1) whether they possessed a mechanism for the recognition of the odour of a predator, and (2) how they responded behaviourally and physiologically to that odour. We used fox, *Vulpes vulpes*, faeces as the source of the predator odour and sheep, *Ovis aries*, faeces as a nonpredator control odour. The experiments were conducted in small outdoor enclosures where the animals were kept singly. We recorded patterns of vigilance, activity and feeding, and changes in glucocorticoids and body mass. The rabbits showed a clear antipredator response to the presence of fox faeces, whereas they behaved neutrally in response to sheep odour. The response consisted of increased avoidance and vigilance while feeding, and more investigation before feeding. Furthermore, the rabbits showed a physiological alarm response, that is, an increased responsiveness of their adrenocortical system and weight loss. However, the total activity budget, measured as time spent outside the burrow, the time spent feeding, and the amount of food ingested remained largely stable during the experiment. We conclude that rabbits recognised predator odours and that this recognition was independent of experience.

INTRODUCTION

Predation is a strong selective force leading to various adaptations in the prey species (Endler 1991; Kats & Dill 1998; Lima 1998a). In addition to adaptations at the morphological, physiological or life history level, animals have evolved a variety of behavioural strategies in order to minimize predation risk. In this context, selection

should have favoured mechanisms to detect predators prior to their attack, increasing the probability of escaping or avoiding encounter.

In addition to the use of, for example, visual, acoustic or vibratory cues, olfactory cues may be important since they may provide information on predation risk even when the predator is absent at the time of detection. The use of these cues may be particularly expected for mammals that have a well-developed chemical sense, such as those which are mainly nocturnal or live in physically complex habitats (Kleiman 1966; Ralls 1971; Johnson 1973; Halpin 1986). Responses of prey species to predator scent have been investigated in many mammals. These studies have focused on different antipredator behaviours such as the direct avoidance of a predator's odour (Dickman & Doncaster 1984; Gorman 1984), changes in feeding behaviour (Ward *et al*. 1997; Blumstein *et al*. 2002; Burwash *et al*. 1998a), variations in space use (Jedrzejewski & Jedrzejewska 1990; Burwash *et al*. 1998b), activity modifications (Jonsson *et al*. 2000; Gorman 1984), and increases in vigilance and alertness (Hennessy & Owings 1978; Caine & Weldon 1989). Nevertheless, there is no general agreement whether animals possess a recognition mechanism that is independent of experience. Even if the behavioural responses are absent, this does not necessarily imply that the animals do not recognize the odour (Ydenberg & Dill 1986). Physiological measures may show that an animal recognizes a predator, but have rarely been used in studies of predator recognition. Under a stressful situation, such as an encounter with a predator, animals show a physiological stress response. For example glucocorticoids increase to mobilize energy which can be used in the typical 'fight or flight' response (von Holst 1998; Buchanan 2000; Creel 2001).

We investigated behavioural traits and physiological changes of predator-naïve European rabbits, *Oryctolagus cuniculus*, exposed to the odour of a predator (red fox *Vulpes vulpes*, faeces). Our main aim was to determine whether rabbits recognize predator odours and respond adaptively without any experience of predators. European rabbits are a good example where one might expect the existence of this mechanism, because this species is reported to rely strongly on its olfactory sense (e.g., Mykytowycz 1968; Bell 1980), and should have a high rate of encounters with the odour of a variety of mammalian predators (Jaksic & Soriguer 1981). We considered whether (1) physiological and behavioural responses occur and (2) rabbits can distinguish between the odour of a predator and nonpredator scent, as the two sufficient conditions for the existence of a recognition mechanism. We looked at the behavioural traits of activity,

vigilance and feeding, and at the physiological measures of glucocorticoid level and body mass.

METHODS

The experiments were carried out at the Department of Animal Physiology, University of Bayreuth, Germany. We used adult European rabbits which were about 8 months old and descendants from wild individuals that had been caught at different sites in south Germany in 1984. The rabbits were reared in social groups in a predator-free environment. During the experiments, they were housed individually in outdoor wire mesh enclosures with sandy soil. Digging was inhibited by a wire-mesh layer underneath the sand, and bird netting was used to exclude raptors. Each of these enclosures (360 × 460 cm) contained an artificial concrete burrow consisting of a tube (length: 150 cm, diameter: 20 cm) and a chamber with a removable top (diameter: 60 cm). In total, six of these enclosures were available for the experiments. We covered the wire mesh between the enclosures with gunny sacks to prevent social interactions between the individuals in the different enclosures. To avoid contact with wild predators, the whole area was surrounded by a wall 4 m high. We placed two wooden boxes (30×30×30 cm) within each enclosure as feeding sites for the rabbits. One side of the box was left open, so the rabbits could enter easily. In each box, we placed two concrete feeding dishes. The outer bowl contained pellet food (Solikanin Plus, Ovator, Germany), and in the inner bowl we placed the scent that was going to be tested on the rabbits. Since the food and the faeces were placed in different bowls, we prevented any contamination of the food with heterospecific faecal compounds. Within each enclosure, the feeding boxes were 3 m apart and 3 from the burrow, forming an equilateral triangle. Water was provided ad libitum in the centre of the enclosure.

In each of the enclosures, we installed a video camera by which we could observe the whole area. During the experiment, we videotaped the animals continuously during each 24-h period using time-lapse recorders. To enable us to record continuously, we fixed a red lightbulb (40 W) in each enclosure, which was automatically switched on during the night.

Sources of Odour

We used faeces from the red fox as the predator odour, and faeces from domestic sheep *Ovis aries* as the heterospecific non-predator odour. Both, sheep and fox faeces were collected from captive animals in Hof Zoological Park, Franconia, Germany. The fresh samples were wrapped inside aluminium foil and were put in plastic bags before being frozen at –20°C. The samples were defrosted shortly before use and soaked in water to reinforce the odour.

Experimental Procedure

We used 11 rabbits (seven females, four males) for our experiment and a further 11 (two females, nine males) as a control group for the physiological measures (see below). However, owing to a camera failure, behavioural data were available from only ten animals in the experiment. The project was approved by the government of Middle Franconia, Germany.

The animals weighed 1308-1802 g before and 1267-1813 g after the experiments. They showed body mass changes of between –8.1% and +4.6% during the experiment and control procedures, which is well below the changes in body mass of European rabbits under natural conditions (cf. Rödel 2000, in press). None of the females were either pregnant or lactating and none of the animals showed signs of ill health during the procedure. We planned to intervene if a rabbit stopped feeding for more than two days; however, the rabbits did not reduce their food intake (see Results). After the experiment, the rabbits were successfully released into their original groups. All females reproduced and at least one male reached the dominant rank position of its group within a few days.

The rabbits were placed into the enclosures 14 days before the experiment to allow them to acclimatize to the new environment. We ensured that all individuals fed from both feeding boxes. At the end of this acclimatization phase, we started the experiment which consisted of three trials. During each of these trials, we videotaped the animals, and recorded their food intake from each of the two feeding boxes per enclosure. Each trial lasted 4 days and there was 1 day between trials; the whole experiment lasted 14 days.

During the first trial, we provided only food without any heterospecific odour. This was done to obtain information on the rabbits' normal activity and food intake. During the next two trials, the rabbits were confronted with heterospecific odours. To

avoid bias from possible preferences for a particular box, every rabbit was recorded for 2 days; we moved the boxes (including the bowl with the odour) between recording days and averaged the scores registered during both trials.

During the second trial, we tested whether the rabbits react to a heterospecific "innocuous" stimulus. This was done to test for novelty effects. We therefore presented sheep faeces in one of the boxes, which was chosen at random. In the alternative box, we placed faeces belonging to the resident rabbit of the enclosure.

In the third trial, we tested for the effects of the presence of predator odour. We therefore presented red fox faeces in one of the boxes, and the heterospecific nonpredator odour (sheep) in the other. Here, the feeding box with sheep odour represented the alternative choice.

Data Collection

All the trials were video recorded and we continuously analysed the videos. We noted the occurrence and the exact duration (with the aid of the internal timer of the video recorders) of different behavioural traits. In particular, we looked at the total activity budget and the time allocated to feeding per day. We also considered vigilance while feeding and avoidance behaviour before the start of feeding.

The total activity per individual was defined as the time spent outside the burrow. Thus, this measure reciprocally represents the refuge use of the rabbits. We calculated the time spent feeding by summing the duration of all feeding events per day, where a feeding event was defined as the period from when a rabbit started to feed until it stopped feeding and left the proximity of the box. This measure was calculated separately for feeding at each of the two boxes present in each enclosure.

As a measure of vigilance, we used the proportion of feeding events (per day) where the animals showed any signs of scanning for a potential predator, such as raising the head, turning around, looking at both sides, looking back or standing on the hind legs. All these behaviours were shown outside the box, and as a consequence they were easily detectable by the observer. We also measured the frequency of avoidance behaviours (per day) before the animals started feeding. More specifically, we counted how many times the rabbits directly approached the feeding box, sniffed at the box, which was apparent by the head movement, but backed away without feeding.

We also measured the food intake. Every morning, a total of 150 g of food pellets were placed in each enclosure, 75 g in each of the two feeding boxes. This

amount exceeded the food requirements of rabbits (cf. Bini & Xiccato 1998). We weighed the remaining food 24 h later, and calculated the food intake from each feeding box.

At the beginning and at the end of the experiment we measured the rabbits' physiological stress response. The measurement of an animal's physiological stress response by means of blood hormone titres entails several methodological problems. These measures are only reliable when animals can be captured rapidly, and blood sampling occurs within a short and standardized period. Furthermore, hormone concentrations in the blood or in the serum provide information only about the animal's present state of stress. An appropriate alternative is to measure serum glucocorticoid levels after an ACTH challenge test (cf. Faulborn et al 1979). The injection of a high dose of synthetic ACTH results in a maximum increase in serum glucocorticoids levels. This maximum level can be interpreted as the adaptive state of an individual's adrenocortical system, and therefore retrospectively provides information about the animal's physiological stress response over several days (von Holst 1998). Another advantage of this test procedure is that the levels are not affected by capture and handling procedures that are performed shortly before measurements are taken.

In rabbits, the injection of a solution of synthetic ACTH increases blood glucocorticoid concentrations (cortisol and corticosterone), which reach a maximum plateau between 60 and 90 min after injection (cf. von Holst 1998; von Holst et al. 1999). Therefore, we caught the rabbits at the beginning (after the habituation period) and at the end of the experiment, injected them intramuscularly with 0.1 ml of synthetic ACTH solution (Synacthen Depot, Novartis, 1 mg/ml), and placed them separately in gunny sacks. After 60 minutes, we took blood samples (300 µl) from the marginal ear veins by puncture with a sterile needle. The rabbits were closely monitored during the whole procedure, and also during the first hour after releasing them into the enclosures. No adverse effects of the procedure were noted. Blood was immediately centrifuged twice and the serum was frozen at –70ºC until analysis. We measured the challenge values of corticosterone in the samples by using a radioimmunoassay (Foster & Dunn 1974). Inter and intra-assay coefficients of variation (CV) were 4.0% and 5.3%. For our analysis, we calculated the percentage change in the serum corticosterone levels after ACTH challenge in relation to the initial level at the beginning of the experiment. We ensured that there were no statistical differences between the initially measured corticosterone serum levels of the rabbits used for the fox/sheep odour experiment and

those in the control group (*t* test for independent samples: $t_{20} = -1.211$, $P = 0.240$). See below for a detailed description of the experiment and the control groups.

We also weighed the animals at the beginning and at the end of the experiment, because body mass is expected to decline under high stress levels (von Holst 1998; Boonstra *et al*. 1998). The rabbits had an average body mass of 1545 ±30 g at the beginning of the experiment, and there were no apparent statistical differences between the initial body mass of the rabbits used for the fox/sheep odour experiment and those in the control group ($t_{20} = -1.521$, $P = 0.145$).

To exclude a possible additive effect of the trials, which could culminate in a strongest response in the last trial (i.e. by the increasing number of stimuli provided), we incorporated a control group into the experiment. Furthermore, this group allowed us to test for the effects of handling, housing and human interference as well as the effects of novelty on the physiological response. For the control group, 11 rabbits were housed in the same conditions as the experimental animals and handled in the same way. They underwent the ACTH challenge test at the beginning and at the end of the control trials and we measured the changes in body mass. However, the groups (experimental and control) differed in the odours presented. The odours were the same as in the experimental rabbits for trial 1 and 2 (own odour and sheep+own odour respectively) but no fox faeces were provided during the third trial. Instead, we presented sheep faeces without providing any other option (i.e., sheep faeces next to both feeding dishes). If rabbits distinguished between predator (fox) odour and heterospecific but nonpredator (sheep) odour, we predicted a lower physiological response in this group.

Data Analysis

All statistical analyses were performed in SPSS for Windows version 11.5 (SPSS Inc., Chicago, IL, U.S.A.). When using parametric test statistics, we ensured that the data were normally distributed (Shapiro-Wilk test), and that variances were homogenous (Levene test). For within-subjects comparisons of the data collected during the three trials of the experiment, we performed Bonferroni-corrected paired *t* tests and Wilcoxon tests post hoc to ANOVA for repeated measures and to Friedman ANOVA, respectively. We therefore used the corrected level of significance $\alpha' = \alpha/k = 0.017$, where α is the error level of 5%, and k is the number of possible comparisons ($k=3$). All tests were two-tailed.

RESULTS

Activity and Feeding

There were no statistically significant differences in the daily time that the rabbits spent outside their warrens (ANOVA for repeated measures: $F_{2,27} = 1.084$, $P = 0.36$) or the time spent feeding ($F_{2,27} = 0.124$, $P = 0.88$) between the three trials of the experiment. On average, we observed the rabbits outside their warrens for 10 h and 6 min ± 87 min per day. During this time, the rabbits fed for on average 72 ± 6 min, representing 12.9 ± 1.0% of their time spent outside the warren. We also did not find any effects of trial (trial 1: no heterospecific odour, trial 2: sheep odour, trial 3: sheep+fox odour) on the rabbits' food intake ($F_{2,30} = 0.088$, $P = 0.92$). On average, the animals ingested 67 g (±4 g SE) of their pellet diet per day.

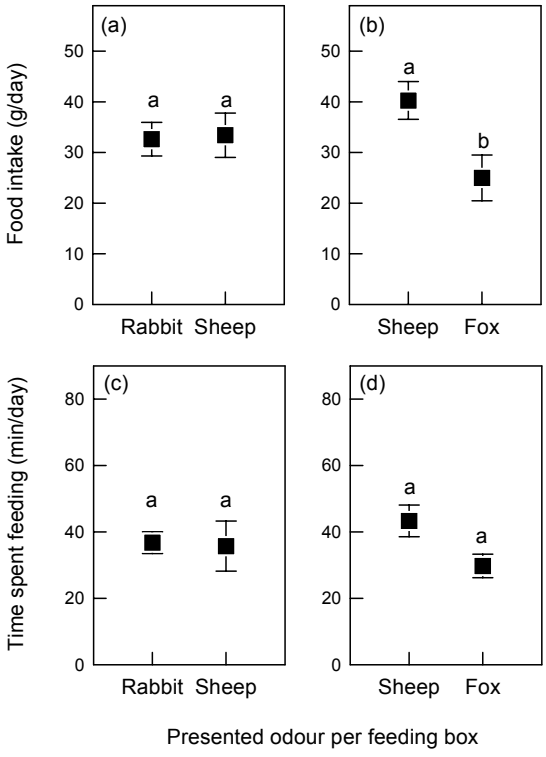

Figure 1. (a, b) Food intake and (c, d) time spent feeding of the rabbits in the two feeding boxes during (a, c) the second (sheep+rabbit odour) and (b, d) the experimental trials (fox+sheep odour). Means are depicted ± SEs. Different letters over the data points show statistically significant differences between groups according to paired *t* tests (see text for statistics).

During the second trial of the experiment, when we placed sheep faeces in one of the feeding boxes and rabbit faeces in the other, neither the amount of food ingested (paired t test: $t_9 = 0.127$, $P = 0.90$; Fig. 1a) nor the time the animals spent feeding ($t_9 = 0.125$, $P = 0.90$; Fig. 1c) differed between feeding boxes. However, we found differences in the third trial of the experiment, when fox faeces were present in one box and sheep faeces in the other. The rabbits ingested significantly less food from the box with fox faeces, on average 32.7% less, compared to the box with sheep faeces ($t_9 = 2.595$, $P = 0.027$; Fig. 1b). The same tendency, although not statistically significant ($t_9 = 1.923$, $P = 0.087$), was apparent for the time spent feeding (Fig. 1d). On average, the animals spent 18.6% less time feeding in the box with fox faeces than in that with sheep faeces.

Vigilance

Vigilance remained stable in the first and second trials of the experiment, but increased significantly during the third trial when one of the feeding boxes was scented with fox odour (ANOVA for repeated measures: $F_{2,27} = 8.689$, $P = 0.002$; post hoc results are depicted in Fig. 2).

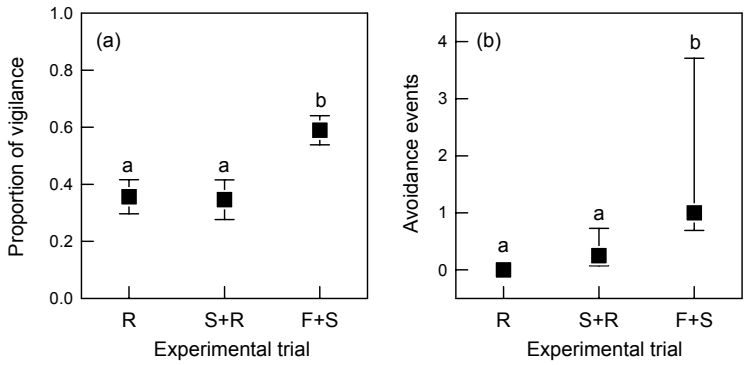

Figure 2. (a) Observed proportion of feeding events where the animals showed vigilance behaviour and (b) the frequency of avoidance events before they started feeding. (a) Means ± SEs and (b) medians with 95% confidence intervals were calculated over each trial of the experiment, during which different combinations of specific or nonspecific faecal odour were presented next to the feeding dishes (F=fox, R=rabbit, S=sheep). Different letters over the data points show statistically significant differences between groups (a) according to a Bonferroni-corrected paired t test post hoc to a repeated measures ANOVA, and (b) to a Bonferroni-corrected Wilcoxon test post hoc to a Friedman ANOVA (see text for statistics).

In total, vigilance was 23.8% higher in the third trial with fox odour than in the first two trials without fox odour (see Fig. 2a). However, there was also a significant increase of on average 31.2% (not shown) in vigilance at the feeding box provided with sheep odour between the second and third trial (Paired *t* test: $t_9 = -3.183$, $P = 0.011$).

Avoidance

This behaviour never occurred during the first experimental trial when no odour was present, and occurred in only a few cases during the second trial, when one box was provided with sheep faeces (Fig. 2b). In total, the frequency of this behaviour significantly increased between the second and the third trial, when in addition to sheep odour, fox odour was present in the alternative feeding box (Friedman ANOVA: $\chi^2_{2,27} = 15.235$, $P < 0.001$; post hoc results are depicted in Fig. 2b). However, the number of avoidance events at the feeding box with sheep odour did not change significantly between the second and third experimental trial (Wilcoxon test: $T = 2.5$, $N = 10$, $P = 0.17$).

Physiological stress response

The percentage changes in corticosterone serum levels differed significantly between the animals of the experimental (including the trial with fox odour) and the control rabbits (*t* test for independent samples: $t_{20} = 2.945$, $P = 0.008$; see Fig. 3a). Rabbits exposed to fox odour showed a distinct increase in corticosterone serum levels of on average 21.8%, whereas the levels of the control animals remained stable.

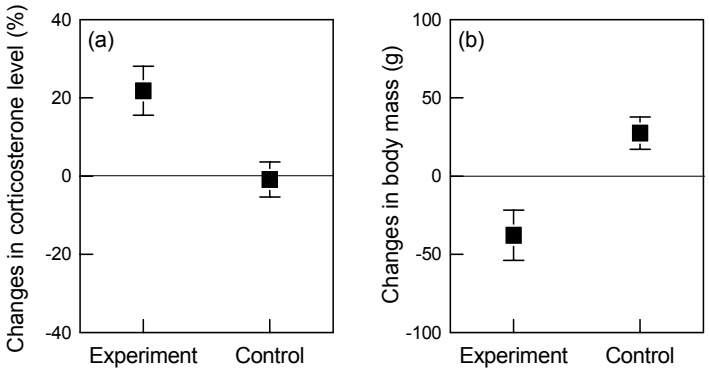

Figure 3. (a) Mean proportional changes (±SE) in corticosterone serum level (after ACTH challenge), and (b) mean absolute changes in body mass during the experimental (rabbit, sheep and fox faeces present; $N = 11$) and control trials (only rabbit and sheep faeces presented; $N = 11$). Different letters show statistically significant differences between groups according to paired *t* tests (see text for statistics).

We also found significant differences in the change in body mass between the groups ($t_{20} = 3.411$, $P = 0.003$; see Fig. 3b). The experimental rabbits lost weight (37 ± 16 g), whereas control rabbits gained weight (28 ± 10 g).

DISCUSSION

Our results strongly suggest that European rabbits possess a mechanism for the recognition of fox odour that is independent of experience. Both conditions were fulfilled: (1) the rabbits showed behavioural as well as physiological responses to the presence of fox odour, but (2) were neutral to heterospecific nonpredator (sheep) odour which we presented as an alternative. In particular, we observed behavioural responses to fox odour in terms of an increase in vigilance and avoidance. Furthermore, there was a significant effect in the stress response, shown by an increase in corticosterone challenge levels and a decrease in body mass compared to the control animals which were not exposed to fox odour.

Both the experimental and the control rabbits had had no previous contact with foxes or sheep. Therefore, the responses we observed were independent of previous experience as also shown in other studies (Boag & Mlotkiewicz 1994; Ward *et al*. 1997; Pongrácz & Altbäcker 2000). However, under natural conditions various effects of learning might modulate this response (Griffin *et al*. 2000; Blumstein *et a*l. 2002; Kavaliers *et a*l. 2003). Nolte *et al*. (1994) suggested that there is a general, nonspecific, carnivorous odour which prey are able to assess as a perilous signal. Sulphurous compounds, a by-product of the digestion of animal proteins, are thought to be responsible for this shared odour. Further evidence comes from the fact that many prey species respond adaptively to the odour of nonendemic predators with which they have never been in contact (Stoddart 1982; Calder & Gorman 1991; Barreto & Macdonald 1999, but see Blumstein 2002).

Our results support these findings. Naïve rabbits were able to distinguish between predator and heterospecific nonpredator chemical cues, avoiding food with fox odour in favour of food with sheep odour (Fig. 1). Furthermore, the absence of an increase in vigilance and avoidance between the first experimental trial without any additional odour, and the second trial with sheep odour provided further evidence that the behavioural responses of the rabbits were not affected by the novelty of this cue (Fig.

2). The observed direct avoidance of a food source or a site with the scent of a predator is a common result of studies focusing on anti-predator responses (reviewed in Kats & Dill 1998) and, as seen in our study, it always leads to a reduction in food intake from this particular food source (e.g., Epple *et a*l. 1993; Boag & Mlotkiewicz 1994; Ward *et al*. 1997; Barreto & Macdonald 1999). Some studies (Kats & Dill 1998) suggested that the scent of a predator may have repelled the prey because of the pungent odour. Nevertheless, in our case, approximately half of the rabbits started feeding in the box with fox odour although they showed more avoidance behaviour in this box than in the alternative sheep box. Thus, a pure repellent effect could be partially excluded, since this should have led to total avoidance.

Rabbits showed more avoidance before they started feeding during the trial when predator odour was present. This implies they postponed feeding. This fact could explain the results of other studies which reported a reduction in the total food intake (e.g., Epple et al. 1993). In these studies, food intake was only recorded over a very short period and the delay in the start of the feeding was not considered. Nevertheless, direct avoidance of food very close to predator faeces is probably of little relevance for the total food gain of an animal, since a herbivore grazer such as the rabbit does not exclusively rely on limited and patchy food resources.

In addition to a greater avoidance, we observed a striking increase in the proportion of feeding events where the rabbits were vigilant. In the third trial, the rabbits more frequently backed away from the dish while feeding, in order to scan the environment. Furthermore, our results indicate that the rabbits generally increased their vigilance when a predator odour was present, independently of the effective cue in the particular feeding box, since we even observed an increase in vigilance at the alternative box provided with sheep odour between the second (without fox odour) and third (with fox odour) experimental trials. Even we were able to perceive the odour of the fox from outside the fence of the enclosure. We therefore assume that the carnivore odour was detectable for the rabbits everywhere in the enclosure and not only within the feeding box provided with fox faeces. An increase in vigilance in response to the presence of predator odour has also been reported in other studies on mammals (Roberts 1988; Caine & Weldon 1989).

Even though rabbits increased their scanning rate and showed avoidance to the odour of a predator, we did not find a reduction in food intake during the experiment. A stable food intake under a simulated predation risk has already been observed in other

studies (e.g., Jones & Dayan 2000). Lima and Bednekoff (1999) suggested that animals under frequent and long-lasting periods of high predation risk might have no other options but to feed. In our experiment, however, the sudden appearance of the predator cue in close proximity should represent an abrupt and high increase in the predation risk (Lima 1998b). Moreover, our rabbits were not nutritionally deprived during the experiment; body condition might affect the balance of the potential trade off between antipredator response and food gain, in this case towards greater antipredator effort (Bachman 1993). Nevertheless, the rabbits did not modify their food intake. Many studies have considered that vigilance behaviour and feeding are mutually exclusive (cf. Bednekoff & Lima 1998). In accordance with Bednekoff & Lima (1998), however, we believe that this assumption might not be generally applicable to all animal species. For example, some studies suggest that birds may routinely manipulate food with their heads up, and they might also be vigilant with their heads down (Bednekoff & Lima 1998). Similarly, during our experiment as well as during behavioural field studies on European rabbits (H. G. Rödel, unpublished data), we frequently observed that they continued chewing while raising their heads and looking around.

Another strategy of predator avoidance could be reduced activity, which is often associated with a reduction in space use. This behaviour decreases the probability of being detected or encountered by a potential predator (e.g., Daly *et al*. 1990; Martel & Dill 1995), and has been reported in many studies on mammals in response to the presence of predator odour (e.g., Dieterlen 1959; Cattarelli 1982; Gorman 1984; Jedrzejewski & Jedrzejewska 1990). Nevertheless, in our study we did not observe any changes in the rabbits' activity patterns in response to the presence of sheep odour or fox odour: the total time spent outside the burrow as well as the time spent feeding remained largely stable. We suspect that the absence of a response in the time spent feeding might be a specific feature of animals which display alarm signals and have burrow systems providing shelter against most predator attacks, such as the European rabbit (Kaetzke et al. 2003). Furthermore, rabbits occupy relatively small home ranges around their warrens (e.g., Cowan 1987; Monclús & Miguel 2003) which may enable them rapidly to escape from a direct threat by a predator. However, we cannot exclude the possibility that under natural field conditions the rabbits might change their space use, for example, by shortening the distance to the entrance of their warren, or they might reduce other activities.

Our result indicating recognition of predator scent independently of experience was supported by the physiological stress response of the animals shown by the increase in corticosterone serum levels and the decrease in body mass during the experiment. In the presence of danger, behavioural and physiological activities are redirected towards immediate life-saving actions (Holberton & Able 2000). The activation of the hypothalamic-pituitary-adrenal axis by an unpredictable stressor leads to gluconeogenesis (von Holst 1998; Buchanan 2000; Creel 2001), and may also result in a lost of weight (cf. Scheuerlein *et al*. 2001). This energy could be used for the 'fight or flight' response.

Few studies have explored the physiological stress response to the recognition of predators by their scent (e.g., Vernet-Maury *et al*. 1984) and these have mostly used the presence of real or stuffed predators to measure the physiological responses (Blanchard *et al*. 1998; Eilam *et al* 1999; Cockrem & Silverin 2002). In most cases, the presence of the predators elicited an increase in the glucocorticoids levels. However, Cockrem and Silverin (2002) found that great tits, *Parus major*, in captivity developed a corticosterone response to stuffed Tengmalm's owl, *Aegolius funereus*, whereas when free-living great tits were tested, no increase in the corticosterone values was apparent. This could be explained by the fact that the presence of a physiological alarm response may depend on the animal's perception of the risk of predation and on the predictability of the stressor as well as on the possible control that it could exert over the situation (Sapolsky 1992; Boissy 1995). In our experiment, rabbits could control the situation to some extent, as they had a refuge (the burrow), but their area of activity was restricted. Apart from this, the rabbits could not predict the potential attack once they had detected the predator odour (Boonstra *et al*. 1998). The lack of these two factors, control and predictability, could have enforced the observed increase in the corticosterone levels (cf. Wolff 2003).

In our experimental design, we exposed the rabbits to an increasing number of heterospecific stimuli. The underlying reason for this was that during the second trial of the experiment, we wanted to assess the effects of heterospecific nonpredator (sheep) odour, and during the last trial, we aimed to compare the effects of nonpredator (sheep) and predator (fox) odour on the rabbits' feeding behaviour. However, our result that the rabbits responded more during the last trial (Fig. 2) cannot be attributed to the increasing number of stimuli (i.e. to a bias from our study design). First, the rabbits did not show any increase in the behavioural parameters between trial 1 and 2. Second, they showed a

strong physiological stress response during the experiment with fox odour but not during the control procedure, where there was the same increase in heterospecific stimuli but no fox odour (see Fig. 3).

Therefore, because of our standardised study set-up including external and internal controls, we were able to show that the recognition of predator odour by European rabbits is independent of experience, and we could assess how they respond to this cue. We speculate that this form of predator avoidance might generally represent a low cost strategy for generalist animals, as could be seen by the absence of any modifications of activity or feeding behaviour. Nevertheless, antipredator responses might be strongly modified under more complex conditions. Therefore, further research should be done such as behavioural and physiological studies under field conditions.

Acknowledgements

We are grateful to Wolf Stöhr and Frank Uhl, who gave us essential technical support, and to Inge Zerenner-Fritzsche who analysed the blood samples. The manuscript was improved by helpful comments from Lutz Dawils, Adrienne Hogg, Cornelia Kraus, Diego Gil and three anonymous reviewers. R. M. was supported by an FPI scholarship from the Comunidad Autónoma de Madrid, Spain.

REFERENCES

Bachman, G. C. 1993. The effect of body condition on the tradeoff between vigilance and foraging in Belding's ground squirrels. *Animal Behaviour*, **46**: 233–244.

Barreto, G. R. & Macdonald, D. W. 1999. The response of water voles, *Arvicola terrestris*, to the odours of predators. *Animal Behaviour*, **57**: 1107–1112.

Bednekoff, P. A. & Lima, S. L. 1998. Randomness, chaos and confusion in the study of antipredator vigilance. *Trends in Ecology and Evolution*, **13**: 284–287.

Bell, D. J. 1980. Social olfaction in lagomorphs. *Symposium of the Zoological Society, London*, **45**: 141–164.

Bini, R. P. & Xiccato, G. 1998. Energy metabolism and requirements. *In*: de Blas, C. & Wieseman, J. (Eds.). *The nutrition of the rabbit*, pp. 103–122. New York: CABI Publishing.

Blanchard, R. J., Nikulina, J. N., Sakai, R. R., McKittrick, C., McEwen, B. & Blanchard, D. C. 1998. Behavioral and endocrine change following chronic predatory stress. *Physiology & Behavior*, **63**: 561–569.

Blumstein, D. T. 2002**.** Moving to suburbia: ontogenetic and evolutionary consequences of life on predator-free islands. *Journal of Biogeography*, **29**: 685–692.

Blumstein, D. T., Mari, M., Daniel, J. C., Ardron, J. G., Griffin, A. S. & Evans, C. S. 2002. Olfactory predator recognition: wallabies may have to learn to be wary. *Animal Conservation*, **5**: 87–93.

Boag, B. & Mlotkiewicz, J. A. 1994. Effect of odor derived from lion faeces on behavior of wild rabbits. *Journal of Chemical Ecology*, **20**, 631–637.

Boissy, A. 1995. Fear and fearfulness in animals. *The Quarterly Review of Biology*, **70**: 165–191.

Boonstra, R., Hik, D., Singleton, G. R. & Tinnikov, A. 1998. The impact of predator-induced stress on the snowshoe hare cycle. *Ecology Monographs*, **79**: 371–394.

Buchanan, K. L. 2000. Stress and the evolution of condition-dependent signals. *Trends in Ecology and Evolution*, **15**: 156–160.

Burwash, M. D., Tobin, M. E., Woolhouse, A. D. & Sullivan, T. P. 1998a. Laboratory evaluation of predator odors for eliciting an avoidance response in roof rats (*Rattus rattus*). *Journal of Chemical Ecology*, **24**: 49–66.

Burwash, M. D., Tobin, M. E., Woolhouse, A. D. & Sullivan, T. P. 1998b. Field testing synthetic predator odors for roof rats (*Rattus rattus*) in Hawaiian Macadamia nut orchards. *Journal of Chemical Ecology*, **24**: 603–630.

Caine, N. G. & Weldon, P. J. 1989. Responses by red-bellied tamarins (*Saguinus labiatus*) to fecal scents of predatory and non-predatory neotropical mammals. *Biotropica*, **21**: 186–189.

Calder, C. J. & Gorman, M. L. 1991. The effects of red fox *Vulpes vulpes* faecal odours on the feeding behaviour of Orkney voles *Microtus arvalis*. *Journal of Zoology, London*, **224**: 599–606.

Catarrelli, M. 1982. The role of the medial olfactory pathways in olfaction: Behavioral and electrophysiological data. *Behavioural Brain Research*, **6**: 339–364.

Cockrem, J. F. & Silverin, B. 2002. Sight of a predator can stimulate a corticosterone response in the great tit (*Parus major*). *General and Comparative Endocrinology*, **125**: 248–255.

Cowan, D. P. 1987. Aspects of the social organisation of the European rabbit (*Oryctolagus cuniculus*). *Ethology*, **75**: 197–210

Creel, S. 2001. Social dominance and stress hormones. *Trends in Ecology and Evolution*, **16**: 491–497.

Daly, M., Wilson. M., Behrends, P. R. & Jacobs, L. F. 1990. Characteristics of kangaroo rats, *Dipodomys merriami*, associated with differential predation risk. *Animal Behaviour*, **40**: 380–389.

Dickman, C. R. & Doncaster, C. P. 1984. Responses of small mammals to red fox (*Vulpes vulpes*) odour. *Journal of Zoology, London*, **204**: 521–531.

Dieterlen, F. 1959. Das Verhalten des syrischen Goldhamsters (*Mesocritecus auratus* Waterhouse). *Zeitschrift für Tierpsychologie*, **16**: 47–103.

Eilam, D., Dayan, T., Ben–Eliyahu, S., Schulman, I., Shefer, G. & Hendrie, C. A. 1999. Differential behavioural and hormonal responses of voles and spiny mice to owl calls. *Animal Behaviour*, **58**: 1085–1093.

Endler, J. A. 1991. Interaction between predators and prey. *In*: Krebs, J. R. & Davies N. B. (Eds.). *Behavioural Ecology – An Evolutionary Approach. 3rd edn.*, pp. 169–196. Oxford: Blackwell Science.

Epple, G., Mason, J. R., Nolte, D. L. & Campbell, D. L. 1993. Effects of predator odors on feeding in the mountain beaver (*Aplodontia rufa*). *Journal of Mammalogy*, **74**: 715–722.

Faulborn, K. W., Fenske, M., Pitzel, L. & König, A. 1979. Effects of an intravenous injection of tetracosactid on plasma corticosteroid and testosterone levels in unstressed male rabbits. *Acta Endocrinologica*, **91**: 511–518.

Foster, L. B. & Dunn, R. T. 1974. Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clinical Chemistry*, **20**: 365–368.

Gorman, M. L. 1984. The response of prey to stoat (*Mustela erminea*) scent. *Journal of Zoology, London*, **202**: 419–423.

Griffin, A. S., Blumstein, D. T. & Evans, C. S. 2000. Training captive-bred or translocated animals to avoid predators. *Conservation Biology*, **14**: 1317–1326.

Halpin, Z. T. 1986. Individual odours among mammals: origins and functions. *Advances in the Study of Behavior*, **16**: 39–70.

Hennessy, D. F. & Owings D. H. 1978. Snake species discrimination and the role of olfactory cues in the snake-directed behaviour of the California ground squirrel. *Behaviour*, **65**: 115–124.

Holberton, R. L. & Able, P. A. 2000. Differential migration and an endocrine response to stress in wintering dark-eyed juncos (*Junco hyemalis*). *Proceedings of the Royal Society of London B*, **267**: 1889–1896.

Jaksic, F. M. & Soriguer, R. C. 1981. Predation upon the European rabbit (*Oryctolagus cuniculus*) in Mediterranean habitats of Chile and Spain: a comparative analysis. *Journal of Animal Ecology*, **50**: 269–281

Jedrzejewski, W. & Jedrzejewska, B. 1990. Effect of a predator's visit on the spatial distribution of bank voles: experiments with weasels. *Canadian Journal of Zoology*: **68**: 660–666.

Johnson, R. P. 1973. Scent marking in mammals *Animal Behaviour*, **21**, 521–535.

Jones, M. & Dayan, T. 2000. Foraging behavior and microhabitat use by spiny mice *Acomys cahirinus* and *A. russatus* in the presence of Blandford's fox (*Vulpes cana*) odor. *Journal of Chemical Ecology*, **26**: 455–469.

Jonsson, P., Koskela, E. & Mappes, T. 2000. Does risk of predation by mammalian predators affect the spacing behaviour of rodents? Two large–scale experiments. *Oecologia*, **122**: 487–492.

Kaetzke, P., Niedermeier, J. & Masseti, M. 2003. *Oryctolagus cuniculus* (Linné, 1758) – Europäisches Wildkaninchen. *In*: Niethammer, J. & Krapp, F. (Eds.). *Handbuch der Säugetiere Europas*, pp. 187–289. Wiebelsheim: Aula Verlag.

Kats, L. B. & Dill, L. M. 1998. The scent of death: chemosensory assessment of predation risk by prey animal. *Ecoscience*, **5**: 361–394.

Kavaliers, M., Colwell, D. D. & Choleris, E. 2003. Learning to fear and cope with a natural stressor: individually and socially acquired corticosterone and avoidance responses to biting flies. *Hormones and Behavior*, **43**: 99–107.

Kleiman, D. 1966. Scent marking in the Canidae. *Symposium of the Zoological Society London*, **18**: 167–177.

Lima, S. L. 1998a. Nonlethal effects in the ecology of predator-prey interactions. What are the ecological effects of anti–predator decision–making? *BioScience*, **48**: 25–34.

Lima, S. L. 1998b. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive and ecological perspectives. *Advances in the Study of Behavior*, **27**: 215–290.

Lima, S. L. & Bednekoff, P. A. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *The American Naturalist,* **153**: 649–659.

Martel, G. & Dill, L. M. 1995. Influence of movements by coho salmon (*Onchorhynchus kisutch*) parr on their detection by common mergansers (*Mergus merganser*). *Ethology*, **99**: 139–149.

Monclús, R. & Miguel, F. J. 2003. Distribución espacial de las letrinas de conejo (*Oryctolagus cuniculus*) en el Monte de Valdelatas (Madrid). *Galemys,* **15**: 157–165.

Mykytowycz, R. 1968. Territorial marking by rabbits. *Scientific American*, **218**: 116–126.

Nolte, D. L., Mason, J. R., Epple, G., Aronov, E. & Campbell, D. L. 1994. Why are predator urines aversive to prey? *Journal of Chemical Ecology*, **20**: 1505–1516.

Pongrácz, P. & Altbäcker, V. 2000. Ontogeny of the responses of European rabbits (*Oryctolagus cuniculus*) to aerial and ground predators. *Canadian Journal of Zoology*, **78**: 655–665.

Ralls, K. 1971. Mammalian scent marking. *Science*, **171**: 443–449.

Roberts, S. C. 1988. Social influences on vigilance in rabbits. *Animal Behaviour*, **36**: 905–912.

Rödel, H. G. 2000. Low temperature effects and social influences on physiological condition of subadult wild rabbits. *In*: Heldmaier, G. & Klingenspor, M. (Eds). *Life in the Cold*, pp. 511–518. Berlin: Springer Verlag.

Rödel, H. G. in press. Winter feeding behaviour of European rabbits in a temperate zone habitat. *Mammalian Biology*, doi: 10.1016/ j.mambio.2005.03.001

Sapolsky, R. M. 1992. Neuroendocrinology of the stress-response. *In*: Becker, J. B., Breedlove, S. M. & Crews, D. (Eds.). *Behavioural Endocrinology*, pp. 287–324. Cambridge: The MIT Press.

Scheuerlein, A., van't Hof, T. J. & Gwinner, E. 2001. Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). *Proceedings of the Royal Society of London B*, **268**: 1575–1582.

Stoddart, M. 1982. Does trap odour influence estimation of population size of the short–tailed vole, *Microtus agrestis*? *Journal of Animal Ecology*, **51**, 375–386.

Vernet–Maury, E., Polak, E. H. & Demael, A. 1984. Structure-activity relationship of stress–inducing odorants in the rat. *Journal of Chemical Ecology*, **10**: 1007–1018.

von Holst, D. 1998. The concept of stress and its relevance for animal behavior. *Advances in the Study of Behavior*, **27**: 1–131.

von Holst, D., Hutzelmeyer, H., Kaetzke, P., Khaschei, M. & Schönheiter, R. 1999. Social rank, stress, fitness, and life expectancy in wild rabbits. *Naturwissenschaften*, **86**: 388–393.

Ward, J. F., Macdonald, D. W. & Doncaster, C. P. 1997. Responses of foraging hedgehogs to badger odour. *Animal Behaviour*, **53**: 709–720.

Wolff, J. O. 2003. Laboratory studies with rodents: facts or artefacts? *BioScience*, **53**: 421–427.

Ydenberg, R. C. & Dill, L. M. 1986. The economics of fleeing from predators. *Advances in the Study of Behavior*, **16**: 229–249.

CAPÍTULO 3

El olor del zorro incrementa la vigilancia de los conejos: un estudio en condiciones seminaturales.

Manuscrito original del artículo publicado en Ethology:

Monclús, R., Rödel, H. G., von Holst, D. 2006. Fox odour increases vigilance in European rabbits: a study under semi-natural conditions. Ethology, 112: 1186 - 1193.

El olor del zorro incrementa la vigilancia de los conejos: un estudio en condiciones seminaturales.

Resumen

El reconocimiento de los olores de los depredadores es un mecanismo conocido en muchas especies de presas que puede conducir a diversas respuestas comportamentales y fisiológicas. Esto se ha demostrado en muchas especies de mamíferos en condiciones de laboratorio, pero los esfuerzos para validar esos resultados en el campo a menudo han conducido a resultados rebatibles. Investigamos las reacciones comportamentales y la respuesta fisiológica de estrés de los conejos (*Oryctolagus cuniculus*) a los olores de un depredador (el zorro, *Vulpes vulpes*) en condiciones seminaturales. El estudio se llevó a cabo en una población de conejos que viven en una instalación exterior de 2 hectáreas. Comparamos las tasas de vigilancia y de exploración, el tiempo dedicado a comportamientos de mantenimiento, los tamaños de las áreas de campeo y las respuestas fisiológicas de un grupo experimental y de un grupo control. Sólo los animales del grupo experimental estuvieron en contacto con heces de zorro. Estos animales aumentaron la tasa de vigilancia, mientras que no se produjeron modificaciones en los animales control. No se encontraron diferencias en la respuesta exhibida por los individuos adultos y los subadultos. Además, los animales experimentales se acercaron con frecuencia al olor del depredador, lo que podría indicar un aumento del comportamiento de investigación. El tamaño del área de campeo, la alimentación y otros comportamientos de mantenimiento no variaron en respuesta al olor del zorro. Asimismo, no hubo diferencias entre los animales del grupo experimental y control en las concentraciones de corticosterona en suero (medido tras una inyección de hormona adrenocorticotropa), medidas antes y después del experimento. Sugerimos que las respuestas comportamentales observadas representan una estrategia de bajo coste para reducir el riesgo individual de depredación.

Fox odour increases vigilance in European rabbits: a study under semi-natural conditions

Abstract

The recognition of predator odours is a well-known mechanism in many prey species which may lead to various behavioural and physiological responses. This has been shown for many mammal species under laboratory conditions, but efforts to validate the results in the field often have led to inconclusive results. We investigated the behavioural reactions and the physiological stress response of European rabbits (*Oryctolagus cuniculus*) to the odour of a mammalian predator (red fox, *Vulpes vulpes*) under semi-natural conditions. The study was conducted on a rabbit population living in an outdoor enclosure of 2 ha. We compared the rates of vigilance and exploration, the time allocated to self-directed behaviours, the home range sizes and the physiological responses of an experimental and a control group. Only animals from the experimental group were confronted with fox faeces. These animals increased their vigilance rate whereas the control animals did not respond. The increase did not differ between adult and subadult individuals. Furthermore, the experimental animals frequently approached the odour of the predator which might indicate an increase in investigative behaviour. Home range size, feeding and other self-directed behaviours did not change in response to fox odour. Moreover, the animals of the experimental and the control group did not differ in serum corticosterone concentrations (measured after adrenocorticotrophic hormone challenge) that we determined in the beginning and in the end of the experiment. We suggest that the observed behavioural responses represent a low-cost strategy for lowering the individual risk of predation.

INTRODUCTION

It is known that many mammal species are able to detect a potential predator by its scent, and this detection usually elicits the display of a behavioural response in the absence of the predator (reviewed in Lima & Dill 1990; Kats & Dill 1998; Apfelbach *et al.* 2005). Such an early response is highly beneficial, because prey animals that detect the predator but remain

undetected have higher chances of a successful escape. Many studies have dealt with the detection of predators by their scent (Hennessy & Owings 1978; Gorman 1984; Caine & Weldon 1989; Jedrzejewski & Jedrzejewska 1990; Ward *et al.* 1997; Burwash *et al.* 1998; Blumstein *et al.* 2002; Woodley & Peterson 2003; Zhang *et al.* 2003). However, most of the experiments have been carried out under laboratory conditions and the few attempts to validate the results under field conditions have led to inconclusive results (Kavaliers & Choleris 2001). Compared with laboratory experiments, the results attained in the field generally have been negative (no responses elicited) or only weakly positive. The stronger responses under laboratory conditions might be attributed to the fact that the animals could not control nor predict the situation. This can result in exaggerated behaviours, which are hardly found under field conditions (Sapolsky 1992; Boissy 1995). On the other hand, factors such as social interactions or energetic demands might mask or modulate the anti-predator responses of animals observed in the wild (e.g. Sullivan *et al.* 2001). Furthermore, factors such as the age of the animals studied, which is often not known under field conditions, might play an important role. Compared with adults, young animals frequently show stronger responses as they also react to unspecific cues (quantitative response hypothesis, Inglis 1979).

In a recent study under laboratory conditions, we showed that European rabbits (Oryctolagus cuniculus) displayed several behavioural and physiological responses when exposed to the odour of a predator, independently of prior experience (Monclús *et al.* 2005). Fox odour provoked an increase in vigilance and avoidance, which was coupled to an increase in serum corticosterone levels, whereas non-predator odour did not elicit any notable behavioural response.

In the present study, we investigated the response of European rabbits to predator odour under seminatural conditions. We conducted our experiments using an individually marked European rabbit population confined to an outdoor enclosure. This enabled us to test the responses to predator odour at the individual level. Firstly, we focused on potential behavioural responses such as vigilance behaviour, spatial avoidance, changes in space use and the time allocated to different self-directed activities, and looked at differences in these responses between young and adult animals. Secondly, we tested for the presence of a

physiological stress response to predator odour by measuring changes in serum corticosterone concentrations.

METHODS

Study Animals and Housing Conditions

The study was conducted in a fenced population of European rabbits kept in an outdoor enclosure of 2 ha near the University of Bayreuth (Franconia, Germany). This population descended from wild individuals that had been caught in 1984. The genetic variability of our study population was within the ranges of wild populations (Niedermeier 1996). For further details see von Holst *et al.* (2002).

The density of adult rabbits, measured during the breeding season prior to our study was 26 individuals per hectare. The animals were organized in eight social groups which were homogeneously distributed across the enclosure and all the animals could be identified by their individual ear-tags.

The enclosure consisted of homogeneous grassland interspersed with groups of trees and bushes. The whole study site could be observed from two separate look-out towers. With the aid of a grid of wooden sticks, it was possible to determine the position of each animal (±2 m).

During the study year, the access of terrestrial predators to the enclosure was restricted by a double electrical wire (cattle fence), attached to the outer side of the enclosure fence at 0.3 and 1.5 m high. However, birds of prey (in particular the common buzzard *Buteo buteo*) still preyed heavily on juveniles during the first few weeks after these emerged above ground.

Study Design and Timetable

The enclosure was virtually divided in two regions: a control area and an experimental area. These were separated by a buffer area (approx. 70 m wide, exceeding the average diameter of a rabbit's home range). We considered that the buffer area was wide enough as to prevent the odour in the experimental area from reaching the control area. When selecting the different regions, we took into account the existing borders of the social

groups. By doing this, we ensured that the home ranges of single animals did not extend into different regions. The experiments and the animal observations were carried out simultaneously in both regions (experimental and control area) to exclude the effects of environmental factors such as weather. We conducted the experiment during the non-breeding season (October–December), when social interactions between adults are usually low (von Holst *et al.* 1999). In the experimental and control areas, two consecutive trials were carried out: first a control trial and then the experiment itself. During the control period, no predator odour was presented to attain basic values for all the behaviours considered. During the experimental period, the experimental group was exposed to fox odour, whereas in the control group no novel odour was presented. In a previous experiment performed with European rabbits (descendants of wild animals) under laboratory conditions, we could effectively exclude novelty effects: rabbits clearly differentiated between heterospecific predator and non-predator odours, and the latter did not provoke any responses (Monclús *et al.* 2005).

To exclude potential effects of the experimenters while placing the odours, we followed a similar walking pattern in the control area as the one performed in the experimental area. Each period lasted 20 d with an interval of 3 d between both periods.

Odour Presentation

The presence of the predator was simulated with the aid of fresh fox faeces (*Vulpes vulpes*) which were placed on tiles. These odour stations were located every 14 m forming a net. Altogether, there were 30 odour stations. The stations were always situated close to the already existing coloured sticks of the grid system and therefore could be easily seen by the observers. In the control area, we placed the same number of tiles in a similar pattern to control for this novel visual cue. Fresh fox faeces were collected from captive animals from the Zoological Park in Hof/Saale, Germany. All the faeces were wrapped in aluminium foil and frozen until short before use. For the experiment, the scats were stirred in hot water, to homogenize the odour. A small amount of this solution (16 ml) was placed on each tile and the odours were renewed every second day.

Data Collection

Behavioural data

The animals from both groups were observed from two observational towers with the aid of binoculars. One of the towers was located close to the experimental group while the other was next to the control group. Two observers conducted the records simultaneously, each in one tower, shifting their positions every second day to exclude observational bias. The observations were carried out daily, 3 h before dusk, when European rabbits show their main activity (Wallage-Drees 1989).

To measure the behavioural responses of the rabbits, we used two different sampling techniques: focal sampling (continuous recording) and scan sampling (Martin & Bateson 1986). The former was used to register frequencies of different behaviours (described below) and the latter was used to register the spatial data. Continuous recording of different behaviours was conducted on 17 focal animals from the experimental group (10 adult females, seven subadults), and 14 animals (five adult females, nine subadults) from the control group. The subadults were the descendants of that year, and the adults were at least 1 yr old. As in most European rabbit populations in the temperate zones, our study animals did not reproduce within their year of birth.

We did not collect data of adult males but only considered the females of this age class. Every focal animal was observed for 15 min and at least for four times on different days (only once per day). We registered the time allocated to feeding, grooming and resting, and the number of vigilance displays per observation unit. We considered the animals to be vigilant when they showed signs of alertness, such as lifting the ears, raising the head and looking around, independently whether they stopped their normal activity or not. Furthermore, we wrote down the time that the rabbits spent in close proximity (less than 1 m) to the odour station.

We also scanned all subadults and adults of the experimental and the control group. The scans were done four times a day. We noted down the spatial location of each individual using the coordinate system. For the analysis, we only considered those animals from which we collected at least 20 fixes per trial, resulting in a sample size of 10 adult females and 13 subadults in the experimental group and 10 adult females and 19 subadults in the control group.

Based on these fixes, we calculated the areas (minimum convex polygons with 100% of the fixes; Kenward 1987) used by the animals during the first and the second period of the study. To gather more information about the individual changes in space use, we determined the proportion of overlap of the area used during both trials, calculated as the average of the overlap between the first trial and second trial and between the second trial and the first trial.

Physiological data

Corticosterone serum levels were measured twice, at the beginning (before the control period started) and at the end of the study (after the experimental period). To minimize the effects of trapping and handling on the values obtained, we performed an adrenocorticotrophic hormone (ACTH) challenge test (cf. Faulborn *et al.* 1979). The test procedure consisted in an injection of a high dose of synthetic ACTH (Synacthen Depot, Novartis, Nürnberg, Germany, 1 mg/ml) prior to bleeding. The ACTH provokes a maximum increase of serum glucocorticoids levels. This maximum level can be interpreted as the adaptive state of an individual's adrenocortical system, and therefore retrospectively provides information about the animal's physiological stress response over a span of time of several days (von Holst 1998). Another advantage of this test is that glucocotricoid concentrations are not affected by the capture and handling procedures that are performed shortly before the measurements are taken.

In rabbits, it has been shown that the injection of a solution of synthetic ACTH causes an increase in blood glucocorticoid concentrations (cortisol and corticosterone) which reach a maximum plateau between 60 and 90 min after injection (cf. von Holst 1998; von Holst *et al.* 1999). Therefore, the animals were trapped in baited wooden live traps, were injected intramuscularly with 0.1 ml of synthetic ACTH solution, and were stored separately in gunny sacks. After 60 min, we took blood samples (300 µl) from the marginal ear veins by puncture with a sterile needle. Blood was mmediately centrifuged twice and the serum was frozen at –70ºC until analysis. We measured the concentration of corticosterone in the samples by using a radioimmunoassay (Foster & Dunn 1974). Inter and intra-assay coefficients of variation (CV) were 4.0% and 5.3%. For our analysis, we calculated the

percentage change in the serum corticosterone concentrations in relation to the level at the beginning of the experiment.

We restricted this analysis to subadults, as we only managed to get a sufficient number of repeated measures (trapping and re-trapping) from this age class (17 subadults from the control group, and nine subadults from the experimental group).

Data Analysis

We used a doubly controlled design where we compared two independent groups (control group and experimental group), and collected repeated measures during the first (control) and second (experimental) period from individuals of both groups. Doing so, we were able to calculate individual changes between both periods for all the variables considered. These changes were used as response variables. Additionally, we always checked for differences in the initial values (i.e. the values during the first trial) between control group and experimental group. Prior to the use of multivariate parametric statistics, we checked that the distribution of the data was approximately normal (Shapiro–Wilk test) and that variances were homogenous (Levene test). If necessary, response variables were transformed to fit these criteria (Kirkwood & Sterne 2003). For this purpose, the frequency of the initial vigilance behaviour measured was log transformed, and we used power transformations for the changes in vigilance behaviour [$(x + 100)^2$]. All statistical analyses were done with the software package SPSS 12.0 (SPSS Inc., Chicago, IL, USA). We used the software Ranges 6 (Anatrack Ltd, Wareham, UK) for the analysis of the spatial data.

RESULTS

Effects on Vigilance

The initial frequencies of vigilance that the animals showed during the first period did not differ between the control and the experimental group (two-way ANOVA: $F_{1,27} = 1.863$, $P = 0.184$; see Fig. 1a). However, we found significant differences between age classes ($F_{1,27} = 12.457$, $P = 0.002$). Compared with adults, the subadults showed 26.4% less events

of vigilance per hour. The interaction of age class and group was not significant ($F_{1,27} = 0.096$, $P = 0.759$).

The changes in vigilance between the first and second period of our experiment differed significantly between the groups (two-way ANOVA: $F_{1,27} = 7.081$, $P = 0.013$; Fig. 1b). The values of the animals of the control group increased on average by 4.9%, and the values of the animals of the experimental group increased by 71.3%. There were no statistically significant differences in the change of vigilance between age classes ($F_{1,27} = 1.411$, $P = 0.245$) and also no significant interaction between both factors ($F_{1,27} = 0.063$, $P = 0.804$).

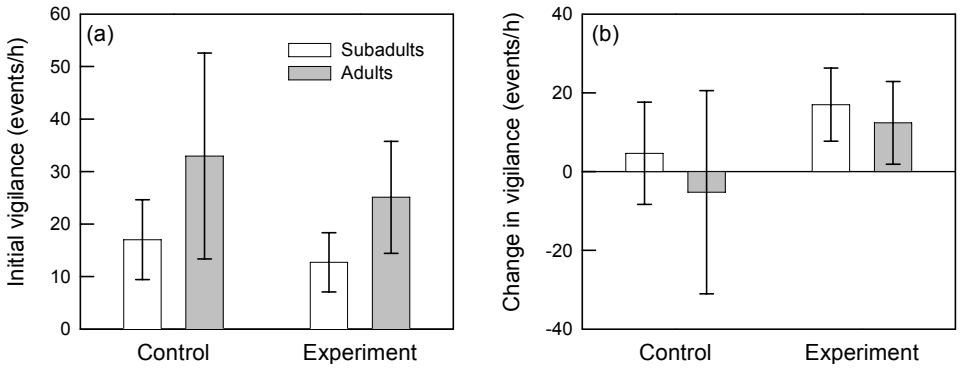

Fig. 1 (a) Comparison between the average initial vigilance (± SD) of animals of the experimental and control group displayed during the first period of the experiment, and (b) between the average changes in vigilance (± SD) in animals of the experimental and control group, shown for subadult and adult individuals. See text for statistics.

Effects on Spacing Behaviour

The estimated home-range size during the first period of the experiment did not differ significantly between the experimental and the control group (two-way ANOVA: $F_{1,36} = 0.365$, $P = 0.549$). We also did not detect any age-specific differences in these initial values ($F_{1,36} = 0.479$, $P = 0.493$). However, the interaction between both factors was significant ($F_{1,36} = 4.755$, $P = 0.036$), indicating the tendency that the home-range size of subadults were slightly higher than that of adults in the control group but slightly lower than that of adults in the experimental group. When confronted to fox odour, the changes in the average size of the area used by the rabbits from the experimental group did not differ

significantly from that of the control group (two-way ANOVA: $F_{1,36} = 0.033$, $P = 0.856$). We also did not detect significant differences between adults and subadults ($F_{1,36} = 0.071$, $P = 0.792$), or a significant interaction between group and age class ($F_{1,36} = 0.012$, $P = 0.912$).

However, the presence of fox odour affected the space use of the animals within their home range. During the first period of the experiment, the animals were rarely observed by us near the tiles, where the fox faeces were later presented (experimental group: 0.3 min/h ±1.1 SD, control group: 0 min/h). Differences between the groups (Mann-Whitney U: $Z = -1.584$, $n_{experiment} = 17$, $n_{control} = 14$, $P = 0.113$) and between age classes (Mann-Whitney U: $Z = -1.392$, $n_{subadult} = 15$, $n_{adult} = 16$, P=0.164) were not significant. When exposed to fox odour, the animals of the experimental group showed a significantly higher increase in the time spent in close proximity (next to the tile within a radius of 1 m) to the odour stations, than the control animals where no fox odour was present (two-way ANOVA: $F_{1,27} = 11.736$, $P = 0.002$; Fig. 2). No differences between age classes were apparent ($F_{1,27} = 0.460$, $P = 0.504$), and also the interaction between age class and group was not significant ($F_{1,27} = 0.236$, $P = 0.631$).

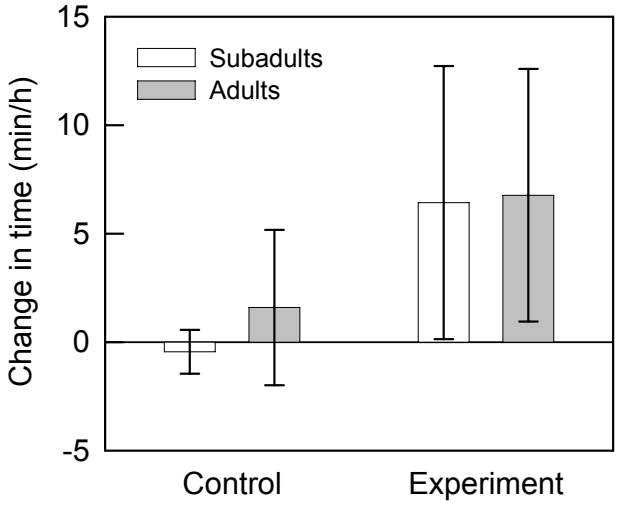

Fig. 2: Comparison between the average changes in the time spent in close proximity to the odour stations (± SD) of animals of the experimental and control group, shown for subadult and adult individuals. See text for statistics

Effects on Self-Directed Behaviours

We did not find any differences between the control and experimental group with respect to the changes in the frequency of feeding (Table 1a), grooming (Table 1b) or resting (Table 1c). The initial values of all response variables also did not differ between the control ($n = 14$) and the experimental group ($n = 17$) (Mann-Whitney U-test; feeding: $Z = -1.212$, $P = 0.225$; grooming: $Z = -1.005$, $P = 0.315$, resting: $Z = -0.080$, $P = 0.936$).

	Response variable	Source of variation	*df*	*F*	*P*
(a)	Time spent feeding	*Group*	1	1.242	0.275
		Age class	1	0.516	0.479
		Group×age class	1	0.435	0.515
		Error	27		
(b)	Time spent grooming	*Group*	1	0.438	0.514
		Age class	1	0.055	0.816
		Group×age class	1	0.151	0.701
		Error	27		
(c)	Time spent resting	*Group*	1	0.665	0.422
		Age class	1	1.417	0.244
		Group×age class	1	1.626	0.213
		Error	27		

Table 1 ANOVA models of the effects of group (control group/experimental group) and age class (subadult/adult) on the changes of different behavioural variables (a-c) between the first and the second period of the experiment, when the animals of the experimental group were confronted with fox odour.

Effects on Serum Corticosterone Concentrations

The initial serum corticosterone concentration of subadults, measured prior to the first period of the study, did not differ significantly between control and experimental group (Mann-Whitney U: $Z = -0.172$, $n_{control} = 19$, $n_{experiment} = 9$, $P = 0.863$). In the control group, the mean initial values accounted 64.77 ng/ml ± 23.30 SD and in the experimental group 64.12 ng/ml ± 36.53 SD. We did not find any differences between both groups neither in the absolute changes ($Z = -1.304$, $P = 0.192$) nor in the percentage changes of the corticosterone concentrations ($Z = -0.861$, $P = 0.389$). In the control group, the corticosterone values on average increased by 28.69 ng/ml ± 19.67 SD

(= 48.2% ± 35.94 SD), and in the experimental group, the values on average increased by 18.06 ng/ml±16.69 SD (=49.93%±63.56 SD).

DISCUSSION

The animals showed clear responses to the presence of fox faeces. These responses consisted in an increase of vigilance and fine-scale spatial changes that were independent of the age class of the rabbits. Nevertheless, the adrenocortical reactivity was not altered by the odour.

Such an increase in vigilance in response to the presence of the odour of a predator has also been reported in other studies on mammals (Hennessy & Owings 1978; Caine & Weldon 1989; Apfelbach *et al.* 2005), but has not been shown so far for European rabbits under field conditions. The results obtained in our study validate previous findings on European rabbits studied under laboratory conditions, where the presentation of fox odour caused similar behavioural responses (Monclús *et al.* 2005).

We did not find differences between subadults and adults in the anti-predator response. Even if subadults had lower basal levels of vigilance, the increase did not differ from that shown by adult rabbits. This supports previous findings on the rabbit's anti-predator behaviour. When exposed to stuffed predators, rabbits of different age classes showed similar reactions (Pongrácz & Altbäcker 2000). However, experience also plays an important role in shaping behaviours (Vitale 1989; Griffin *et al.* 2000; Blumstein *et al.* 2002; Kavaliers *et al.* 2003). The quantitative response hypothesis predicts that young animals exhibit stronger reactions but become more selective in their responses as they gain experience (Inglis 1979). In accordance, we would have expected to find age-related differences, i.e. subadults showing stronger responses to fox odour than adults. A possible explanation for their, however, generally lower level of vigilance might be that subadult, and even 1 yr old rabbits, are in lower body condition and show a higher feeding activity than older individuals (Rödel *et al.* 2004; Rödel 2005). This might lead to the observed lower allocation to vigilance in subadults in favour of food intake, as also suggested in other studies (FitzGibbon 1989; Murray 2002).

A second anti-predator behaviour, which European rabbits showed in a study under laboratory conditions was spatial avoidance of the odour (Monclús *et al.* 2005). However, our findings strikingly differed from our expectations: the rabbits under laboratory conditions showed avoidance behaviour against the fox odour, whereas they showed attraction towards these places in the field enclosure. We suggest that the fact that they spent time in close proximity to the odour could be because of an acquisition of information about the predator and the assessment of the risk of predation. Thus, the observed spatial behaviour would represent investigation episodes. Such investigative behaviour has also been described in characin fishes (Brown *et al.* 2000), especially when visual signals were limited. Another example comes from a study on red-bellied tamarins (*Saguinus labiatus*) which visited more and spent more time sniffing the odour stations tainted with predator odour than with non-predator odour (Caine & Weldon 1989). Kats & Dill (1998) hypothesized that areas around fresh predator droppings might constitute safer zones for prey species, as the probability of an encounter is lower because of the spacing pattern of the predators (Ables 1983).

However, on a broader scale, we did not find any spatial modification. The rabbits of our experimental group maintained their individual home-range size and also did not shift their space use in a different way than the animals of the control group. Similarly, Jonsson *et al.* (2000) did not find spatial modifications in response to predator odour in two species of voles in a field experiment. Drastic changes in space use might be too costly for an animal, especially when other anti-predator behaviours are more appropriate in case the predator is still nearby, such as an increase in alertness. Furthermore, the costs and consequences, which can be associated with changing the territory in response to predator cues (i.e. loss of food resources, loss of familiar social environment, loss of the communal warren, etc.) might outweigh the benefits of a decreased predation pressure.

We also did not find changes in the time allocated to self-directed activities such as grooming, resting and feeding. Accordingly, the rabbits did not change their time allocated to feeding in our laboratory experiment (see Monclús *et al.* 2005). Nevertheless, in the present study, we did not measure the food intake, so we could not definitely exclude differences in feeding effort (cf. Lima & Bednekoff 1999).

The presence of fox odour did not provoke a physiological stress response by means of an increase in serum corticosterone concentrations. This finding is in contrast to the results attained by our study on European rabbits under laboratory housing conditions (Monclús *et al.* 2005). One explanation might be that positive findings in laboratory studies might just be artefacts. Caged animals are usually restricted in space and activity, and therefore have low control over their present situation. This might strongly enforce the physiological reaction to a potential stressor (Sapolsky 1992; Boissy 1995; von Holst 1998). In contrast, our study animals were not space limited; the area of the field enclosure (20 000 m2) by far exceeded the average home-range size of a European rabbit (400–500 m^2; Myers & Poole 1959; Parer 1982; own observations). Furthermore, the animals in our study were living in a natural social environment. Many studies on mammals show that the integration in a social network or even the presence of familiar conspecifics can buffer the individual stress response to challenging situations (e.g. DeVries *et al.* 2003). Similar results were found by Cockrem & Silverin (2002) in a study on great tits (*Parus major*). They showed that in captive birds the sight of a stuffed owl increase glucocorticoid concentrations while in free-ranging animals the simulated predator did not.

Nevertheless, Boonstra *et al.* (1998) found effects of predation pressure on the stress response in wild snowshoe hares (*Lepus americanus*). Animals living under high risk of predation had higher glucocorticoid levels than hares under a milder risk. Therefore, another explanation for the absence of such a response in our study might be the lack of reinforcement by a direct encounter with a predator under our semi-natural conditions.

In conclusion, our study shows that European rabbits living under natural conditions react to the odour of a mammalian predator. The anti-predator strategy consisted of low-cost behaviours which might facilitate an effective avoidance of the predator. However, there are still several issues that should be addressed, as for example how an animal's sex, reproductive state and body condition modulates these responses.

Acknowledgements

We would like to thank Christfried Kurz for the field observations and Inge Zerenner-Fritzsche who analysedthe blood samples. We are also grateful toAnett Starkloff, Barbara Bruchner, Manuela Zapka and Christine Geier for their support in the field work, and to Florian Lengyel for correcting the English text. Permission for the animal experiments was granted by the government of Middle Franconia (211-3894a; 621-2531.32-5/05).

LITERATURE CITED

Ables, E. D. 1983. Ecology of the red fox in America. *In*: Fox, M. W. (Ed.). *The Wild Canids. Their Systematics, Behavioral Ecology and Evolution*, pp. 216–236. Florida: Robert E. Krieger Publishing Company, Inc.

Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A. y McGregor, I. S. 2005. The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neuroscience and Biobehavioral Reviews*, **29**: 1123–1144.

Blumstein, D. T., Mari, M., Daniel, J. C., Ardron, J. G., Griffin, A. S. & Evans, C. S. 2002: Olfactory predator recognition: wallabies may have to learn to be wary. Animal Conservation, **5**: 87–93.

Boissy, A. 1995. Fear and fearfulness in animals. *The Quarterly Review of Biology*, **70**: 165–191.

Boonstra, R., Hik, D., Singleton, G. R. & Tinnikov, A. 1998: The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs*, **79**: 371–394.

Brown, G. E., Paige, J. A. & Godin, J. G. J. 2000: Chemically mediated predator inspection behaviour in the absence of predator visual cues by a characin fish. Animal Behaviour, **60**: 315–321.

Burwash, M. D., Tobin, M. E., Woolhouse, A. D. & Sullivan, T. P. 1998: Laboratory evaluation of predator odours for eliciting an avoidance response in roof rats (*Rattus rattus*). Journal of Chemical Ecology, **24**: 49–66.

Caine, N. G. & Weldon, P. J. 1989: Responses by red-bellied tamarins (*Saguinus labiatus*) to fecal scents of predatory and non-predatory neotropical mammals. *Biotropica*, **21**: 186–189.

Cockrem, J. F. & Silverin, B. 2002: Sight of a predator can stimulate a corticosterone response in the great tit (*Parus major*). General and Comparative Endocrinology **125**: 248–255.

DeVries, A. C., Glasper, E. R. & Detillion, C. E. 2003: Social modulation of stress responses. Physiology & Behavior, **79**: 399–407.

Faulborn, K. W., Fenske, M., Pitzel, L. & König, A. 1979: Effects of an intravenous injection of tetracosactid on plasma corticosteroid and testosterone levels in unstressed male rabbits. *Acta Endocrinologica*, **91**: 511–518.

FitzGibbon, C. D. 1989: A cost to individuals with reduced vigilance in groups of Thomson's gazelles hunted by cheetahs. *Animal Behaviour*, **37**: 508–510.

Foster, L. B. & Dunn, R. T. 1974. Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clinical Chemistry*, **20**: 365–368.

Gorman, M. L. 1984. The response of prey to stoat (*Mustela erminea*) scent. *Journal of Zoology London*, 202: 419–423.

Griffin, A. S., Blumstein, D. T. & Evans, C. S. 2000. Training captive-bred or translocated animals to avoid predators. Conservation Biology, **14**: 1317–1326.

Hennessy, D. F. & Owings, D. H. 1978. Snake species discrimination and the role of olfactory cues in the snakedirected behaviour of the California ground squirrel. *Behaviour*, **65**: 115–124.

von Holst, D. 1998: The concept of stress and its relevance for animal behaviour. *Advances in the Study of Behavior*, **27**: 1–131.

von Holst, D., Hutzelmeyer, H., Kaetzke, P., Khaschei, M. & Schönheiter, R. 1999. Social rank, stress, fitness, and life expectancy in wild rabbits. *Naturwissenschaften*, **86**, 388–393.

von Holst, D., Hutzelmeyer, H., Kaetzke, P., Khaschei, M., Rödel, H. G. & Schrutka, H. 2002. Social rank, fecundity and lifetime reproductive success in wild European rabbits (*Oryctolagus cuniculus*). *Behavioral Ecology and Sociobiology*, **51**, 245–254.

Inglis, I. R. 1979. Visual bird scorers an ethological approach. *In*: Wright, E. N., Inglis, I. R. & Feare, C. J. (Eds.). *Bird Problems in Agriculture*, pp. 121–143. London: MAFF Publishers.

Jedrzejewski, W. & Jedrzejewska, B. 1990. Effect of a predator's visit on the spatial distribution of bank voles: experiments with weasels. *Canadian Journal of Zoology*, **68**: 660–666.

Jonsson, P., Koskela, E. & Mappes, T. 2000. Does risk of predation by mammalian predators affect the spacing behaviour of rodents? Two large-scale experiments. *Oecologia*, **122**: 487–492.

Kats, L. B. & Dill, L. M. 1998. The scent of death: chemosensory assessment of predation risk by prey animal. *Ecoscience*, **5**: 361–394.

Kavaliers, M. y Choleris, E. 2001. Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neuroscience and Biobehavioral Reviews*, **25**: 577–586.

Kavaliers, M., Colwell, D. D. & Choleris, E. 2003. Learning to fear and cope with a natural stressor: individually and socially acquired corticosterone and avoidance responses to biting flies. *Hormones and Behavior*, **43:** 99–107.

Kenward, R. 1987. *Wildlife Radio Tagging: Equipment, Field Techniques and Data Analysis*. London: Academic Press.

Kirkwood, B. & Sterne, J. 2003. *Essential Medical Statistics*. Oxford: Blackwell Publishing.

Lima, S. L. & Bednekoff, P. A. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. American Naturalist, **153**: 649–659.

Lima, S. L. & Dill, L. M. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**: 619–640.

Martin, P. & Bateson, P. 1986. *Measuring Behaviour*. Cambridge: Cambridge University Press.

Monclús, R., Rödel, H. G., von Holst, D. & de Miguel, J. 2005. Behavioural and physiological responses of naïve rabbits to predator odour. *Animal Behaviour*, **70**: 753–761.

Murray, D. L. 2002. Differential body condition and vulnerability to predation in snowshoe hares. *Ecology*, **71**: 614–625.

Myers, K. & Poole, W. E. 1959. A study of the biology of the wild rabbit, *Oryctolagus cuniculus*, in confined populations. The effects of density on home range and the formation of breeding groups. *CSIRO Wildlife Research*, **4**: 14–26.

Niedermeier, J. 1996. Populationsgenetische Untersuchungen an Europaïschen Wildkaninchen (*Oryctolagus cuniculus* L.): Multilocus DNA-Fingerprinting mit chemiluminiszenter Signaldetektion. PhD Thesis, Univ. of Bayreuth, Bayreuth, Germany.

Parer, I. 1982. Dispersal of the wild rabbit, *Oryctolagus cuniculus*, at Urana in New South Wales. *Australian Wildlife Research*, **9**: 427–441.

Pongrácz, P. & Altbäcker, V. 2000. Ontogeny of the responses of European rabbits (*Oryctolagus cuniculus*) to aerial and ground predators. *Canadian Journal of Zoology*, **78**: 655–665.

Rödel, H. G. 2005. Winter feeding behaviour of European rabbits in a temperate zone habitat. *Mammalian Biology*, **70**: 300–306.

Rödel, H. G., Bora, A., Kaiser, J., Kaetzke, P., Khaschei, M. & von Holst, D. 2004. Density-dependent reproduction in the European rabbit: a consequence of individual response and age-dependent reproductive performance. *Oikos*, **104**: 529–539.

Sapolsky, R. M. 1992. Neuroendocrinology of the stress response. *In*: Becker, J. B., Breedlove, S. M. & Crews, D. (Eds.). *Behavioural Endocrinology*, pp. 287–324. Cambridge: The MIT Press.

Sullivan, A. M., Maerz, J. C. & Madison, D. M. 2001. Anti-predator response of red-backed salamanders (*Plethodon cinereus*) to chemical cues from garter snakes (*Thamnophis sirtalis*): laboratory and field experiments. *Behavioral Ecology and Sociobiology*, **51**: 227–233.

Vitale, A. F. 1989. Changes in anti-predator responses of wild rabbits, *Oryctolagus cuniculus* (L.), with age and experience. *Behaviour*, **110**: 47–61.

Wallage-Drees, M. 1989. A field study on seasonal changes in circadian activity of rabbits. *Zeitschrift für Säugetierkunde*, **51**: 26–36.

Ward, J. F., Macdonald, D. W. & Doncaster, C. P. 1997. Responses of foraging hedgehogs to badger odour. *Animal Behaviour*, **53**: 709–720.

Woodley, C. M. & Peterson, M. S. 2003. Measuring responses to simulated predation threat using behavioural and physiological metrics: the role of aquatic vegetation. *Oecologia*, **136**: 155–160.

Zhang, J. X., Cao, C., Gao, H., Yang, Z. S., Sun, L., Zhang, Z. B. & Wang, Z. W. 2003. Effects of weasel odour on behaviour and physiology of two hamster species. *Physiology & Behavior*, **79**: 549–552.

CAPÍTULO 4

Diferentes formas de vigilancia en respuesta a la presencia de depredadores y congéneres en un animal que vive en grupo, el conejo

Manuscrito original del artículo enviado a Ethology:

Monclús, R., Rödel, H. G. Different forms of vigilance in response to the presence of predators and conspecifics in a group-living mammal, the European rabbit

Diferentes formas de vigilancia en respuesta a la presencia de depredadores y congéneres en un animal que vive en grupo, el conejo

Resumen

En los animales que viven en grupo, las funciones principales de la vigilancia son detectar la presencia de depredadores y controlar los movimientos de los congéneres. La distancia mínima a un competidor que un animal considera segura suele ser menor que a un depredador, mientras que la frecuencia de encuentros con los primeros es mayor. Por lo tanto, la obtención de información sobre los depredadores o sobre los congéneres podría conducir a la existencia de al menos dos formas diferentes de vigilancia.

El propósito del presente estudio fue describir y comparar los tipos de vigilancia que los conejos, *Oryctolagus cuniculus*, desarrollan en contextos antidepredatorios y sociales. Realizamos un estudio con animales individualmente marcados procedentes de una población en una instalación natural. Registramos las interacciones sociales de los animales, la presencia de depredadores aéreos (busardo ratonero *Buteo buteo*), y el comportamiento de vigilancia de los conejos. Diferenciamos dos tipos de vigilancia que diferían en intensidad: vigilancia sutil y conspicua.

Ambas formas de vigilancia que mostradas por los conejos difirieron significativamente en frecuencia de aparición, duración y distribución a lo largo del tiempo. Las hembras y los machos mostraron una frecuencia mayor de vigilancia conspicua, pero no de vigilancia sutil, cuando el busardo estaba presente. Por el contrario, la presencia en proximidad de congéneres afectó al desarrollo de vigilancia sutil pero no de conspicua. Los machos aumentaron la frecuencia de comportamiento sutil cuando otros machos estaban cerca. Las hembras aumentaron la vigilancia sutil cuando los machos y otras hembras estaban en proximidad, sin embargo, este efecto sólo fue evidente en las hembras con una situación social más inestable. En conclusión, los conejos aumentaron diferencialmente dos tipos distintos de vigilancia en contextos sociales y antidepredatorios.

Different forms of vigilance in response to the presence of predators and conspecifics in a group-living mammal, the European rabbit

Abstract

In group-living mammals, the major functions of vigilance are to detect the presence of predators and to control the movements of conspecifics. The minimum distance to a competitor that an animal considers safe is usually lower than to a predator, whereas the frequency of encounters with conspecifics is higher. Therefore, the acquisition of information about a predator or about a conspecific could lead to the existence of at least two different modes of vigilance behaviour.

The aim of the present study was to describe and compare different types of vigilance behaviour that European rabbits, *Oryctolagus cuniculus*, display in anti-predator and social contexts. We conducted a study on individually marked animals from a field enclosure population. We recorded social interactions of the animals, the presence of aerial predators (common buzzard *Buteo buteo*), and the rabbits' vigilance behaviour. We distinguished between two forms of vigilance that differed in intensity: subtle and overt.

The frequencies of both forms of vigilance displayed by the rabbits differed significantly in occurrence, duration, and distribution over time. Females and males showed higher frequencies of overt but not subtle vigilance when buzzards were present. In contrast, the presence of conspecifics in close proximity affected the rabbits' display of subtle but not overt vigilance: Males increased the frequency of subtle vigilance when other males were close. Females increased subtle vigilance in proximity of males and females; however, this effect was only apparent in females with a more unstable social situation. In conclusion, European rabbits differentially increased two different types of vigilance behaviour in social and anti-predator contexts.

INTRODUCTION

The outcome of an interaction between a prey and a predator largely depends on the timing of detection of the predator by the prey. In order to attain information about nearby predators animals are vigilant by scanning the environment and numerous studies have focused on vigilance as an anti-predator behaviour (reviewed in Lima & Dill 1990; Kavaliers & Choleris 2001; European rabbit *Oryctolagus cuniculus*: Roberts 1988; Monclús *et al*. 2005, 2006).

The presence of conspecifics can modify the display of vigilance behaviour of an animal. In fact, many group-living animals show a negative relationship between individual vigilance and group size (Elgar 1989; Roberts 1996; Lima 1998; Treves 2000; Childress & Lung 2003), and the sharing of anti-predator vigilance has been suggested to be a driving force in the group formation in many species (Hamilton 1971).

Nevertheless, living in social networks also carries several costs for the individual. Animals of the same sex frequently compete for resources, and agonistic interactions among them are part of the daily social life in almost all group-living mammals (von Holst 2001). Subordinate individuals or animals in an unstable social situation are prone to attacks by other group members. They should be aware of the presence of conspecifics and as it has been shown in mammals (Renouf & Lawson 1986; Roberts 1988; Blumstein *et al*. 2001; Cameron & du Toit 2005) and birds (Catterall *et al*. 1992; Pravosudov & Grubb 1999), vigilance could also serve a social function.

Generally, an animal's vigilance consists of scanning events of variable length at random intervals, where the animal raises the head and looks around. Feeding activity might be interrupted or not during these scanning bouts (Bednekoff & Lima 1998a; Fortin *et al*. 2004). An animal's scanning behaviour might differ adaptively in the form, depending on the cue provoking its state of alertness. The minimum distance to a predator that an individual considers safe is usually much higher than to a conspecific competitor (Bednekoff & Lima 1998a). For detecting far-away objects, such as predators, animals should increase the duration of the scans, whereas the scan rate might depend on the risk of predation. On the other hand, conspecifics that pose a threat are usually close by and they should require a less conspicuous display of alert behaviour with shorter scans. However, the scans might be more frequent in order to control the movements of potential interaction partners (Roberts 1988).

We conducted a study on the vigilance of individually marked European rabbits from a field enclosure population. Our principal goal was to describe and compare the vigilance of the animals in response to conspecifics and to predator presence. The European rabbit is a good model for the study of vigilance in social and anti-predator context: Rabbits live in social groups organised in sex-specific linear rank orders. During the breeding season, intraspecific aggressive encounters are frequent (Mykytowycz 1959; Cowan 1987a, b; von Holst *et al*. 1999). Furthermore, rabbits display several forms of vigilance that differ in intensity (Monclús *et al*. 2005).

In our study we first tested whether low intense (more subtle) and high intense (more overt) vigilance differed in the frequency and length of the scans, and in the distribution over time. We expected that the rabbits might mainly increase subtle forms of vigilance in social contexts, whereas overt forms of vigilance would mainly serve as anti-predator response. Therefore, we (1) compared at an individual basis the display of both forms of vigilance when conspecifics were in close proximity or not. We additionally considered the stability of the animals' social situation, which might have modified their responses. We (2) compared the display of subtle and overt vigilance by the rabbits when predators were present or not.

MATERIAL AND METHODS

Study population

The study was conducted on animals from a population of European rabbits living in a 20,000-m² field enclosure of the University of Bayreuth (Germany). Vegetation consisted of grassland interspersed with groups of trees and bushes, which represents an adequate habitat structure for the European rabbit (Corbet 1994). In addition to the burrows and breeding stops dug by the rabbits (around 40), the area contained 16 artificial concrete warrens with interconnected chambers and removable tops. These were used by the rabbits as the main warrens of their group territories and for breeding.

The population consisted of descendents of animals that had been caught in the wild (Bavaria, Germany) in 1983. At the onset of the study period in early July 2006, the population consisted of seven different social groups with a total of 23 adult females and 14 males. According to field data, the density in our enclosure was high but still within the range for wild rabbit populations (e.g., Thompson & Worden 1956; Wallage-Drees

& Michielsen 1989; Caruso & Siracusa 2001; Palomares 2001). During our long-term study, we found no signs of inbreeding such as changes in body mass, juvenile survival, or reduced fecundity of females. For further details on the study population see von Holst *et al*. (2002).

All animals were individually marked with aluminium ear-tags and the composition of the social groups was known by prior behavioural observations. A double electric fence prevented terrestrial but not aerial predators from accessing the enclosure. Permission for population biology studies on European rabbits was provided by the government of Middle Franconia (211-3894a).

Behavioural observations

We recorded behavioural data from two outlook towers from where the whole enclosure could be observed. The study was conducted during the mid-late breeding season (mid July to mid October 2006). The breeding season of our population usually started in April and lasted until mid October (Rödel *et al*. 2005). We collected data from 15 adult females and 13 adult males by means of focal sampling techniques (Martin & Bateson 1993). The observations were conducted during the last 3 hours before twilight, when rabbits usually show the peak of their daily activity (Wallage-Drees 1989). The animals were observed while feeding, so any display of vigilance could be unequivocally recorded. We observed every animal for five minutes (continuous recording) in 12 different sessions resulting in a total observation time of one hour per animal, and a total of 336 five-minute observation sessions. These 12 sessions per individual were evenly distributed over the three months of the study. During every five-minute session, we recorded the duration and frequency of any behavioural sign of vigilance using a portable computer with the software OBSERVER (version 3.0 for DOS, Noldus Inc., The Netherlands). We also recorded the occurrence of agonistic interactions and noted down the number and identity of all rabbits within a five-meter radius around the focal animal.

Behavioural variables

Vigilance

We distinguished between two modes of vigilance, depending on the intensity, and named them as follows:

Subtle vigilance: low intensity response; the animal raises the head above shoulder in a quadrupedal posture while looking around, usually without interrupting its feeding activity.

Overt vigilance: high intensity response; the animal adopts an upright posture while looking around, either quadrupedal or bipedal, lifts the ears and stops all current activities (i.e. feeding).

The frequency of occurrence and the length of the scanning bouts were not used as characteristics for distinguishing between both forms of alert behaviour.

Stability of an individual's social situation

The social system of the rabbit is characterised by sex-specific linear rank hierarchies. Females mainly compete with the other females of the group for the access to burrows whereas males compete for the access to the females (Cowan 1987a, b). Intrasexual aggression reaches the maximum at the beginning of the breeding season, when the social ranks are established among the members of the social group (von Holst *et al*. 1999). However, rank hierarchies are not always stable and intrasexual agonistic interactions are common all over the season. Some individuals are in a more unstable social situation than others, and one would expect that animals with unstable social situations should show higher levels of alertness. In order to determine the stability of an individual's social situation, we summed up the number of escalated agonistic interactions that the animal experienced over the 12 five-minute observation sessions, and calculated the total frequency of interactions per hour. The agonistic interactions considered were chasing or being chased by other individuals of the same sex. Ritualized agonistic behaviours such as displacing or being displaced by other animals were not considered. We performed a median cut over all values of the animals of the same sex, and defined animals with values higher than the median as being in an unstable social situation while the social situation of animals with smaller values was considered stable. In females, the frequency of total agonistic interactions ranged between 0 and 10 interactions per hour, and the median was 2. In males, the frequency of agonistic interactions per hour ranged from 0 to 5 interactions per hour and the median was 3.

Proximity of conspecifics

During all five-minute observational sessions, we determined whether the focal animal was in close proximity (i.e. within a five-meter radius) to adult conspecifics or

not. We chose 5 meters because, due to the structural characteristics of the enclosure, animals within that radius were surely in visual contact with the other group members. Five-meter intervals could be assessed by the aid of a grid system made out of wooden sticks, which was fixed all over the study area.

We considered that an individual was in close proximity to the focal animal only if it stayed for more than one minute of the five-minute observational session within a distance of five meters. For each of the 12 observational sessions per individual, the focal animal was assigned to be alone or with conspecifics.

For every focal animal, we considered two different grouping situations that could explain the alert response. We determined whether same sex animals or animals from both sexes in close proximity were correlated with the vigilance elicited.

Based on this, females were considered to be in close proximity to adult conspecifics in 49% of cases and they were in close proximity to other females in 29% of cases. Males were with other conspecifics in 15% of cases whereas they were with other males in 56% of the occasions.

Furthermore, we measured the total number of animals (adults of both sexes plus juveniles) which were in close proximity (< 5 m) to the focal animal. This was done for females as well as for males; this variable is hereafter referred to as group size. In case the number of close conspecifics changed during the five-minute session, we used the mean value of the number of animals present during five one-minute intervals.

Predator presence

The animals of the enclosure population were regularly in contact with wild common buzzards (*Buteo buteo*), which frequently arrived in the late afternoon and circled over the enclosure, or sat on the trees inside the enclosure. Adult rabbits react to buzzards by behavioural signs of alertness but not or hardly to kestrels (*Falco tinnunculus*) or crows (*Corvus corone corone*) (R.M. & H.G.R. pers. obs). We did not observe the presence of other diurnally active aerial predators during our study.

For every five-minute observation session, we recorded whether a buzzard was present or not. In order to determine predator presence, we recorded any visual or auditory signs of the buzzard. Regular scans for buzzards were done before starting the daily observations and every five minutes when changing the focal animal. In total, buzzards were present during 32.2% of the five-minute observation sessions.

Data analysis and sample sizes

Differences between the two types of vigilance

We tested whether the two types of vigilance (subtle or overt vigilance), differed in the frequency and the mean duration of the bouts when the animals showed one of the two behaviours. Furthermore, we compared the coefficient of variation based on the frequencies recorded during the twelve observation sessions of each animal. This measure provides information about the evenness of the distribution of the two different behavioural variables over time.

Effects of predator presence and social factors

The major goal of our study was to test whether the presence of a predator (common buzzard) or different social factors affected the display of the two different behavioural components of the rabbits' vigilance. For this, we only used the frequencies of subtle and overt vigilance as response variables but not the total duration of these two parameters. Frequency and duration were highly collinear for subtle vigilance (averaged values per animal over all 12 observation sessions: $r^2 = 0.700$, $n = 28$, $P < 0.001$) as well as for overt vigilance ($r^2 = 0.706$, $n = 28$, $P < 0.001$). We analysed our data in two steps.

First, we tested whether the presence of a predator explained the display of either subtle or overt vigilance. Therefore, we split the data in two sets: One set consisted of the averaged values measured for each individual when the predator was present. The other set consisted of data from the same individuals when the predator was absent. Using these data, we calculated repeated measurements ANOVAs where we included the factor sex and the interaction of both factors in order to consider sex-specific differences in the response to predator presence (see Fig. 1a). This statistical model was calculated separately for the frequencies of both subtle and overt vigilance. In total, we ran the analysis with the complete set of focal animals ($n_{females} = 15$, $n_{males} = 13$). In a further step, we only considered the data when the predator was present and tested if there was a sex-specific effect of group size on the display of subtle and overt vigilance by ANCOVA. We included the interaction of sex with the covariate group size. If non-significant, the covariate interaction term was removed and the model was recalculated (see Engqvist 2005).

Second, we split the data for each individual with respect to the presence or absence of conspecifics in close proximity (< 5 m distance). We ran the analyses

separately for females and males, since we considered different social contexts (see Figs. 1c, b): For males and females, we considered the presence/absence of other adult conspecifics of the same sex as potential social factors triggering the display of alert behaviour. Moreover, for both sexes we used the presence/absence of conspecifics (adult males and females) in close proximity (Fig. 1c, e). Again, we used repeated measurements ANOVAs and tested for differences between both situations. For both sexes, we used the individual's social situation as fixed factor with two levels (unstable/stable). We always considered the interaction between this factor and the respective repeated measurements of the model (Fig. 1). Eight females were in an unstable situation whereas seven were in a stable social situation. In males, six were in an unstable situation and five were in a stable situation. For this second step, we could only use a lower sample size of males ($n_{males} = 11$), since two of the males were never observed to be in close proximity to another male and were removed from all the analysis described in Fig. 1d, e.

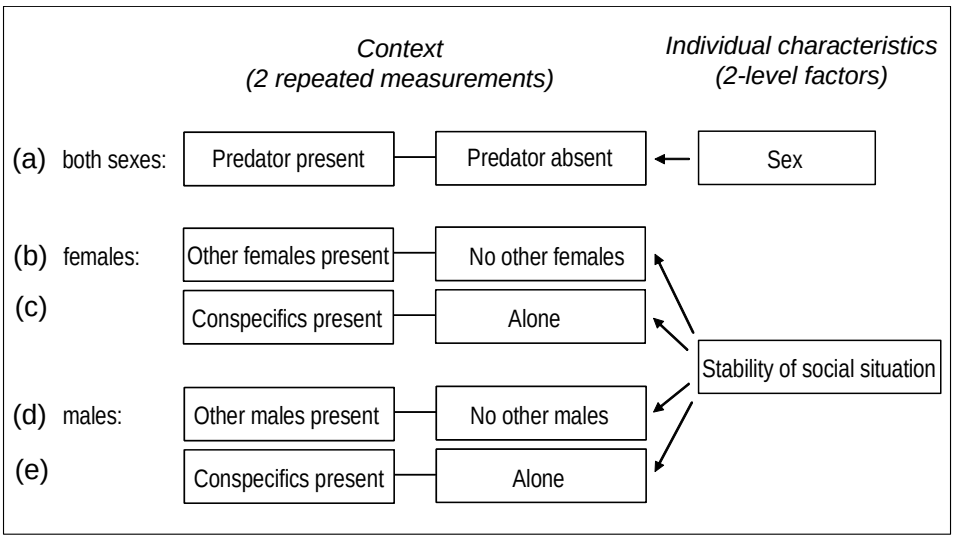

Fig. 1. Outline of the data analysis with respect to (a) the presence of predators (common buzzard) and (b, c, d, e) conspecifics. Response variables are the frequencies of subtle and overt vigilance.

Statistical analysis

Prior to the use of parametric statistics, we ensured that the distribution of the data was approximately normal (Shapiro Wilk test) and that variances were homogenous (Levene test). If these preconditions were not fulfilled the data were transformed. In all

of these cases, we used a log-transformation in order to normalise the right-skewed distribution of the data. Analyses were done with SPSS 14.0 (SPSS Inc. USA).

RESULTS

Differences between two types of vigilance

In our study, we distinguished between two different types of alert behaviour displayed by the rabbits: subtle and overt vigilance.

Both behavioural variables were not correlated significantly ($r = 0.261$, $n = 28$, $P = 0.180$). Compared to overt vigilance, subtle vigilance was displayed in a much higher frequency (paired t-test: $t_{27} = 17.474$, $P < 0.001$; Fig. 2a) and consisted of shorter bouts (Wilcoxon signed rank test: $Z_{27} = -4.532$, $P < 0.001$; Fig. 2b). Furthermore, subtle vigilance was more evenly distributed over time, which was apparent by the comparatively lower coefficient of variation (paired samples t-test: $t_{27} = -8.075$, $P < 0.001$; Fig. 2c).

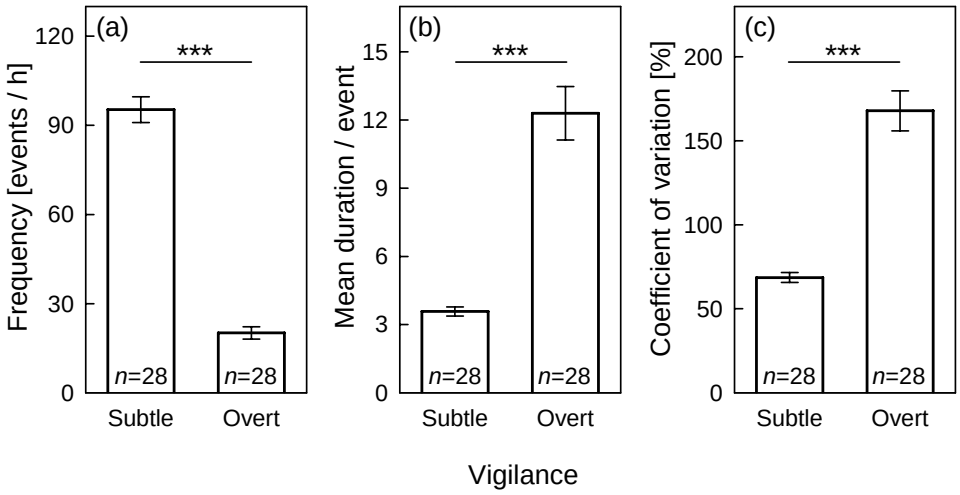

Fig. 2. Comparison of subtle and overt vigilance displayed by adult European rabbits by means of (a) the frequency of occurrence, (b) the mean duration of the bouts and (c) the coefficient of variation. Data (given as means ±SE) represent repeated measurements of the same individuals; sample sizes are shown in the bars. Statistically significant differences are indicated by asterisks; see text for statistics.

The presence of buzzards did not modify the frequency of subtle vigilance displayed by the rabbits (repeated measurements ANOVA: $F_{1,26} = 1.015$, $P = 0.323$; Fig.

3a). There were no significant differences between sexes ($F_{1,26} = 0.352$, $P = 0.558$) and no significant interaction between predator presence and sex ($F_{1,26} = 0.109$, $P = 0.744$).

Overt vigilance

Predator presence significantly increased the frequency of overt vigilance by on average 87% (repeated measurements ANOVA: $F_{1,26} = 15.761$, $P = 0.001$; Fig. 3b). The response did not differ between males and females ($F_{1,26} = 1.214$, $P = 0.281$) and the interaction term predator presence × sex was not statistically significant either ($F_{1,26} = 2.601$, $P = 0.119$).

Only considering the cases when a predator was present, we tested for the effects of group size (i.e., the presence of conspecifics within a distance of five meters) on the frequency of overt vigilance. However, no significant effect of this variable was apparent (ANCOVA: $F_{1,25} = 0.112$, $P = 0.740$) and there were no differences between males and females ($F_{1,25} = 1.812$, $P = 0.190$). The covariate interaction term group size × sex was not significant ($F_{1,24} = 0.638$, $P = 0.432$) and was removed from the final model.

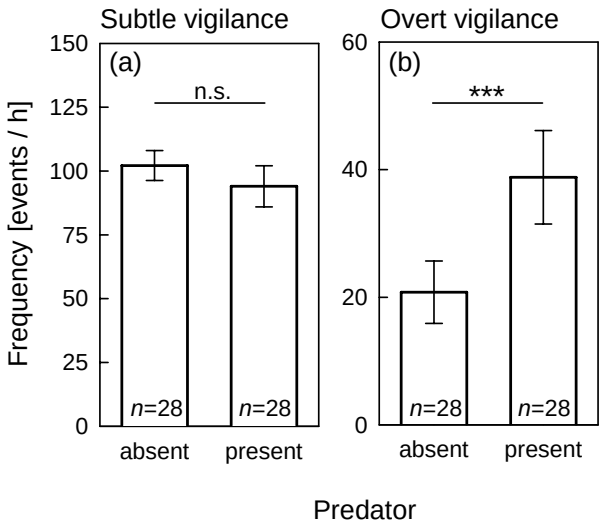

Fig. 3. Effects of the presence of common buzzard on the frequency of (a) subtle and (b) overt vigilance displayed by adult European rabbits. Data (given as means ±SE) represent repeated measurements of the same individuals; sample sizes are shown in the bars. Statistically significant differences are indicated by asterisks; see text for statistics.

Subtle vigilance of females

The proximity of individuals of the same sex did not have a significant effect on the females' display of subtle vigilance, either in individuals with an unstable or stable social situation (Table 1a).

However, the number of adult conspecifics in close proximity affected the display of subtle vigilance, although differently in individuals with a different social situation (see significant interaction in Table 1b). Females, which were in a more unstable social situation showed a significantly higher rate of subtle vigilance when conspecifics were close (paired t-test: $t_7 = -2.405$, $P = 0.047$; Fig. 4a). In contrast, females in a more stable social situation did not show statistically significant differences between both situations (paired t-test: $t_6 = 1.396$, $P = 0.212$; Fig. 4b).

Source of variation		Subtle vigilance		Overt vigilance	
Females		$F_{1,12}$	P	$F_{1,12}$	P
(a)	Proximity to other females	0.002	0.964	1.164	0.300
	Stability of social situation	0.002	0.961	0.006	0.940
	Proximity × stability	1.178	0.297	0.051	0.824
(b)	Proximity to conspecifics	0.061	0.809	0.098	0.760
	Stability of social situation	0.227	0.641	0.152	0.703
	Proximity × stability	**6.636**	**0.023**	0.039	0.847
Males		$F_{1,9}$	P	$F_{1,9}$	P
(c)	Proximity to other males	**7.262**	**0.025**	0.075	0.791
	Stability of social situation	3.152	0.110	1.700	0.225
	Proximity × stability	0.002	0.965	1.979	0.193
(d)	Proximity to conspecifics	1.975	0.194	0.051	0.826
	Stability of social situation	1.289	0.286	0.080	0.784
	Proximity × stability	0.539	0.481	1.829	0.209

Table 1. Effects of different social factors on subtle and overt vigilance of female (a, b: $n = 15$) and male (c, d: $n = 11$) European rabbits (repeated measurements ANOVA). Repeated measurements were taken when the animals were alone or in close proximity (< 5 m) to other adult individuals of the same sex (a, c), or adults of both sexes (b, d). The stability of the individuals' social situation (stable/unstable) is included as a factor. The data for (d) were log-transformed prior to analysis. Statistically significant effects are highlighted in bold letters

Subtle vigilance of males

The frequency of subtle vigilance displayed by males was significantly, about 42%, higher in situations when other males were in proximity compared to situations when no other males were close (Fig. 5a). The stability of the males' social situation did not show any significant effect.

We did not find any significant differences in the males' display of subtle vigilance when considering the presence/absence of adult conspecifics of both sexes (Table 1c).

Overt vigilance of females

Females did not show any differences in the frequency of overt vigilance when comparing situations when other females (Table 1a) or females and males (Table 1b; see Fig. 4c, d) were in close proximity or not.

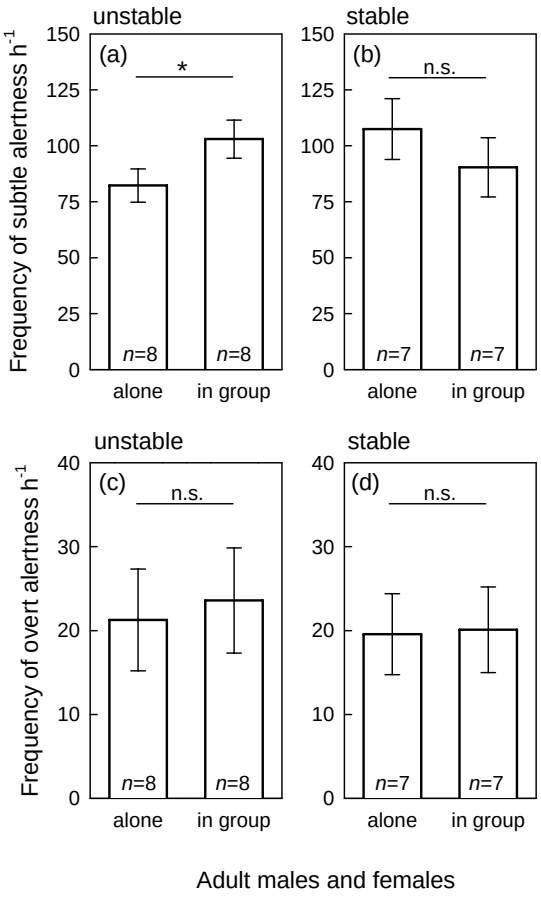

Fig. 4. Effects of the presence of conspecifics in close proximity (< 5 m) on the frequency of subtle (a, b) and overt vigilance (c, d) displayed by adult female European rabbits. Individuals with an unstable (a, c) and stable (b, d) social situation are tested. Data (given as means ±SE) represent repeated measurements of the same individuals; sample sizes are shown in the bars. Statistically significant differences are indicated by asterisks; see text for statistics.

In males, we also did not find any significant effects of the presence/absence of other males (Table 1c; see Fig. 5b) or of adult conspecifics of both sexes (Table 1d) on the display of overt vigilance.

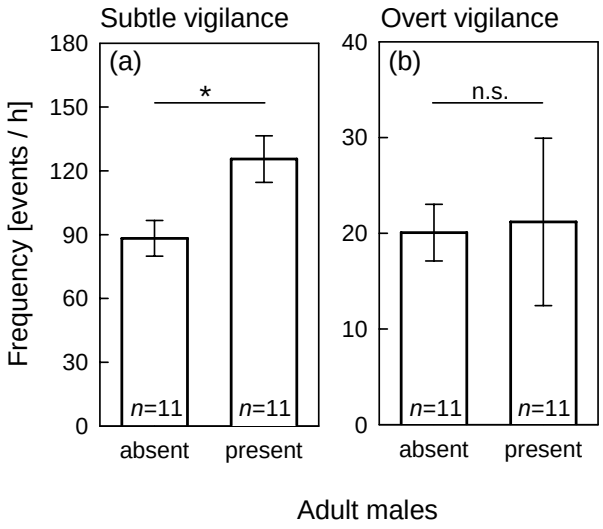

Fig. 5. Effects of the presence of male intruders in close proximity (< 5 m) on the frequency of (a) subtle and (b) overt vigilance displayed by adult male European rabbits. Data (given as means ± SE) represent repeated measurements of the same individuals; sample sizes are shown in the bars. Statistically significant differences are indicated by asterisks; see text for statistics.

DISCUSSION

Several studies suggest that the alert behaviour of an animal could serve several functions (e.g., Renouf & Lawson 1986; Blumstein *et al*. 2001; Tchabovsky *et al*. 2001). In our study, we could show that vigilance in European rabbits consists of at least two components that differ in the frequency and in the length of the display. The presence of close conspecifics and the presence of predators differentially affected the rabbits' display of these two behaviours.

We found differences in the rate, the mean duration, and the distribution over time between subtle and overt alert responses. The former consisted of frequent and short scans, whereas the latter consisted of long and less frequent scans. Therefore, subtle vigilance followed the conditions for monitoring objects in close proximity, allowing an individual to assess small and subtle variations in the position of close-by

individuals. Overt vigilance met the conditions for long distance surveillance, because longer scans are necessary to detect far away objects (Bertram 1980; Roberts 1988).

Both forms of vigilance did not correlate, pointing out that different cues may trigger their display. The further results of our study confirm the adaptive use of the two different behaviours in social and anti-predator contexts: We found clear differences in the rabbits' display of the two types of alert behaviour in situations with/without the presence of predators and with/without close proximity of conspecifics.

When common buzzards were present, rabbits increased the frequency of overt vigilance. Generally, such an increase in scanning rates during situations of increased predation risk has been described in many other studies on rabbits or other mammals (e.g., Caine & Weldon 1989; Lima & Dill 1990; Frid 1997; Monclús *et al*. 2006). However, our results clearly show that only the more overt form of vigilance was increased in response to predator presence. The overt behaviour consisted of the adoption of an upright posture, in many cases bipedal, with the ears up. It also entailed the giving-up of the current activities of the rabbits, such as feeding. These postures could serve different purposes. Above all, the early detection of the spatial location of the predator, including an estimate of the distance, could increase the chances of a successful escape (Endler 1991; Kats & Dill 1998; Lima 1998; Bednekoff & Lima 1998b). Moreover, the vigilance display itself could act as a cue for the predator about the wariness of the animal. In rabbits, the upright postures highlight some physical features that make them very conspicuous to visual predators, such as the long and contrasting-coloured ears (Lockley 1964). Indeed, there is evidence that predators preferably attack less vigilant preys (FitzGibbon 1989), and therefore, the acquisition of an overt alert behaviour could deter predatory attacks (Scannell *et al*. 2001).

Interestingly, we did not find a buffering effect of group size on the display of anti-predator vigilance as exemplified by the lack of a correlation between the number of conspecifics around the focal animal and the frequency of overt vigilance. One possible explanation could be that we only considered cases when the predator was present what constitutes a high-risk situation; under such conditions usually all animals of a group increase vigilance (Lima & Dill 1990).

We did not find sex-specific differences in the overt alert response to predator presence. In contrast, such differences have been reported in black-tailed prairie dogs (*Cynomys ludovicianus*), where males were more vigilant than females (Loughry 1993) and in elks (*Cervus elaphus*) where males were less vigilant (Winnie & Creel 2007).

Due to the observational and semi-natural character of our study, it was not possible to study the animals in situations with conspecifics present versus situations of complete social isolation. Instead, we used the proximity of rabbits within a radius of five meters around the focal animal. We found a clear increase in subtle vigilance when conspecifics were within this distance, however, this response was sex-specific. Male rabbits generally increased their scanning rates when potential competitors were nearby. A similar reaction has been found in other species. For instance, when nine-banded armadillos (*Dasypus novemcinctus*), which are mainly solitary, encountered other individuals, they showed increased levels of vigilance (McDonough & Loughry 1995). Furthermore, giraffe bulls (*Giraffa camelopardalis*) increased scanning when bigger males where in proximity (Cameron & du Toit 2005).

In females, we also found alert responses to close conspecifics. However, the stability of the females' social situation was an important factor in modifying these responses. Females in unstable social situations increased the frequency of subtle vigilance when other rabbits were close, whereas females with a stable social situation did not. In contrast to males, subtle vigilance displayed by females did not differ between situations where animals of the same sex were present or absent; however, we found differences when considering the presence of adult individuals of both sexes. This points out that not only the presence of potential same-sex competitors but also of potential mating partners are important cues for vigilance in females. Female rabbits share space with other females of their group, and agonistic interactions between them are frequent (von Holst *et al*. 1999). The increased number of scan bouts could be due to the need to gather information about the group members (Fernández-Juricic *et al*. 2005), such as their relative spatial position. Based on this information, females might avoid attacks or start them, for example in order to chase away potential infanticidal females from the own breeding burrows (Agrell *et al*. 1998). On the other hand, scanning for present males and gathering information about their movements might also be relevant: especially younger males are sometimes harassing females outside their oestrus by showing courtship behaviour (pers. obs.), and females are usually terminating these apparently unwanted approaches by chasing the young males away.

Apart from differing in the shape and in the eliciting context, both components of vigilance could differ in the costs associated. Subtle vigilance could be considered a low cost behaviour as it did not affect other activities, such as feeding (Lima & Bednekoff 1999; Tchabovsky *et al*. 2001). In those herbivores, where the food resource

is not limited, and their main limitation resides in food handling, the time while processing food could be used for other behaviours, such as vigilance (Illius & Fitzgibbon 1994; Cowlishaw *et al*. 2003; Fortin *et al* 2004). In fact, rabbits handled food while looking for conspecifics, so subtle vigilance and foraging were not exclusive.

However, when the rabbits displayed overt vigilance, which apparently served for anti-predator purposes, they did stop their feeding activity. A study carried out in bison (*Bison bison*) and elk (*Cervus elaphus*) showed that in some occasions the animals stopped chewing while scanning (Fortin *et al*. 2004). The authors suggested that this could be due to the perception of elevated risk. Similarly, the animals in our study stopped their feeding activity when scanning for a predator. Nevertheless, we think that it is not likely that the skip of a few feeding opportunities could entail a notable cost for a grazer such as the rabbit. A further hint comes from experimental studies on European rabbits, where increased rates of vigilance did not affect the animals´ daily food intake (Monclús *et al*. 2005). We rather believe that adult rabbits in healthy body condition are not limited in their daily time budget for feeding, at least during the vegetation period. Similar findings have been reported for golden marmots (*Marmota caudata aurea*; Blumstein 1996).

In conclusion, we recommend taking into account both, the form and the context of the alert responses when studying vigilance behaviour in group-living animals. Only summing up the animals' frequency of scans could lead to incorrect conclusions. Moreover, the social situation of an animal might strongly affect its perceived risk and therefore the display of its alert response.

Acknowledgements

We are grateful to Anett Starkloff who helped with the data collection and to Frank Uhl for technical support. We thank Dietrich von Holst and Javier de Miguel for their valuable comments on earlier drafts of the manuscript. R.M. was supported by a grant from the German Academic Exchange Service DAAD.

REFERENCES

Agrell, J., Wolff, J. O. & Ylönen, H. 1998. Counter strategies to infanticide in mammals: costs and consequences. *Oikos*, **83**: 507–517.

Bednekoff, P. A. & Lima, S. L. 1998a. Randomness, chaos and confusion in the study of antipredator vigilance. *Trends in Ecology and Evolution*, **13**: 284–287.

Bednekoff, P. A. & Lima, S. L. 1998b. Re-examining safety in numbers: interactions between risk dilution and collective detection depend upon predator targeting behaviour. *Proceedings of the Royal Society B*, **265**: 2021–2026.

Bertram B. C. R. 1980. Vigilance and group size in ostriches. *Animal Behaviour*, **28**: 278–286.

Blumstein, D. T. 1996. How much does social group size influence golden marmot vigilance? *Behaviour*, **133**: 1133–1151.

Blumstein, D. T., Daniel, J. C. & Evans, C. S. 2001. Yellow-footed rock-wallaby group size effects reflect a trade-off. *Ethology*, **107**: 655–664.

Caine, N. G. & Weldon, P. J. 1989. Responses by red-bellied tamarins (*Saguinus labiatus*) to fecal scents of predatory and nonpredatory neotropical mammals. *Biotropica*, **21**: 186–189.

Cameron, E. Z. & du Toit, J. T. 2005. Social influences on vigilance behaviour in giraffes, *Giraffa camelopardalis*. *Animal Behaviour*, **69**: 1337–1344.

Caruso, S. & Siracusa, A. M. 2001. Factors affecting the abundance of wild rabbit (*Oryctolagus cuniculus*) in agro-ecosystems of the Mount Etna park. *Hystrix Italian Journal of Mammalogy*, **12**: 45–49.

Catterall C. P., Elgar M. A. & Kikkawa, J. 1992. Vigilance does not covary with group size in an island population of silvereyes (*Zosterops lateralis*). *Behavioral Ecology*, **3**: 207–210

Childress, M. J. & Lung, M. A. 2003. Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour*, **66**: 389–398.

Corbet, G. B. 1994. Taxonomy and origins. *In*: Thompson, H. V. & King, C. M. (Eds.). *The European rabbit. The history and biology of a successful colonizer*, pp. 1–7. Oxford: Oxford University Press.

Cowan, D. P. 1987a. Group living in the European rabbit (*Oryctolagus cuniculus*): mutual benefit or resource localization? *Journal of Animal Ecology*, 56: 779–795.

Cowan, D. P. 1987b. Aspects of the social organisation of the European rabbit (*Oryctolagus cuniculus*). *Ethology*, **75**: 197–210.

Cowlishaw, G., Lawes, M. J., Lightbody, M., Martin, A., Pettifor, R. & Rowcliffe, J. M. 2003. A simple rule for the costs of vigilance: empirical evidence from a social forager. *Proceedings of the Royal Society B*, **271**: 27–33.

Elgar, M. A. 1989. Predator vigilance and group size in mammals and birds: a critical review of the empirical evidence. *Biological Reviews*, **64**: 13 – 33.

Endler, J. A. 1991. Interactions between predators and prey. *In*: Krebs, J. R. & Davies, N. B. (Eds.). *Behavioural ecology: An evolutionary approach*, pp. 169–196. Oxford: Blackwell Scientific.

Engqvist, L. 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour*, **70**: 967–971.

Fernández-Juricic E., Smith, R. & Kacelnik, A. 2005. Increasing the costs of conspecific scanning in socially foraging starlings affects vigilance and foraging behaviour. *Animal Behaviour*, **69**: 73–81.

FitzGibbon, C. D. 1989. A cost to individuals with reduced vigilance in groups of Thomson's gazelles hunted by cheetahs. *Animal Behaviour*, **37**: 508–510.

Fortin, D., Boyce, M. S., Merrill, E. H. & Fryxell, J. M. 2004. The costs of vigilance in large mammalian herbivores. *Oikos*, **107**: 172–180.

Frid, A. 1997. Vigilance by female Dall's sheep: interactions between predation risk factors. *Animal Behaviour*, **53**: 799–808.

Hamilton, W. D. 1971. The geometry of the selfish herd. *Journal of Theoretical Biology*, **31**: 295–311.

Illius, A. W. & Fitzgibbon, C. 1994. Costs of vigilance in foraging ungulates. *Animal Behaviour*, **47**: 481–484.

Kats, L. B. & Dill, L. M. 1998. The scent of death: chemosensory assessment of predation risk by prey animal. *Ecoscience*, **5**: 361–394.

Kavaliers, M. & Choleris, E. 2001. Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neuroscience and Biobehavioural Reviews*, **25**: 577–586.

Lima, S. L. 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive and ecological perspectives. *Advances in the Study of Behavior*, **27**: 215–290.

Lima, S. L. & Dill, L. M. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**: 619–640.

Lima, S. L. & Bednekoff, P. A. 1999. Back to the basics of antipredatory vigilance: can nonvigilant animals detect attack? *Animal Behaviour*, **58**: 537–543.

Lockley, R. M. 1964. *The private life of the rabbit*. London: Andre Deutsch (Ltd.).

Loughry, W. J. 1993. Determinants of time allocation by adult and yearling black-tailed prairie dogs. *Behaviour*, **124**: 23–43.

Martin, P. & Bateson, P. 1993. *Measuring behaviour. An introductory guide*. Cambridge: Cambridge University Press.

McDonough C. M. & Loughry W. J. 1995. Influences on vigilance in nine-banded armadillos. *Ethology*, **100:** 50–60.

Monclús, R., Rödel, H. G., von Holst, D. & Miguel, F. J. 2005. Behavioural and physiological responses of naïve European rabbits to predator odour. *Animal Behaviour*, **70**: 753–761.

Monclús, R., Rödel, H. G. & von Holst, D. 2006. Fox odour increases vigilance in European rabbits: a study under semi-natural conditions. *Ethology*, **112**: 1186-1193.

Mykytowycz, R. 1959. Social behaviour of an experimental colony of wild rabbits, *Oryctolagus cuniculus* (L.). II. First breeding season. *CSIRO Wildlife Research*, **4**: 1–13.

Palomares, F. 2001. Comparison of three methods to estimate rabbit abundance in a Mediterranean environment. *Wildlife Society Bulletins*, **29**: 578–585.

Pravosudov, V. V. & Grubb, T. C. 1999. Effects of dominance on vigilance in avian social groups. *The Auk*, **116**: 241–246.

Renouf, D. & Lawson, J. W. 1986. Harbour seal vigilance: Watching for predators or mates? *Biology of Behaviour*, **11**: 44–49.

Roberts, G. 1996. Why individual vigilance declines as group size increases. *Animal Behaviour*, **51**: 1077–1086.

Roberts, S. C. 1988. Social influences of vigilance in rabbits. *Animal Behaviour*, **36**: 905–913.

Rödel, H. G., Bora, A., Kaetzke, P., Khaschei, M., Hutzelmeyer, H., Zapka, M., & von Holst, D. 2005. Timing of breeding and reproductive performance of female European rabbits in response to winter temperature and body mass. *Canadian Journal of Zoology*, **83**: 935–942.

Scannell, J., Roberts, G. & Lazarus, J. 2001. Prey scan at random to evade observant predators. *Proceedings of the Royal Society B*, **268**: 541–547.

Tchabovsky, A. V., Popov, S. V. & Krasnov, B. R. 2001. Intra and interspecific variation in vigilance and foraging of two gerbilid rodents, *Rhombomys opimus* and *Psammomys obesus*: the effect of social environment. *Animal Behaviour*, **62**: 965–972.

Thompson, H. V. & Worden, A. N. 1956. *The Rabbit.* London; Collins New Naturalist.

Treves, A. 2000. Theory and method in studies of vigilance and aggregation. *Animal Behaviour*, **60**: 711–722.

von Holst, D., Hutzelmeyer, H. D., Kaetzke, P., Khaschei, M., Schönheiter, R. 1999. Social rank, stress, fitness, and life expectancy in wild rabbits. *Naturwissenschaften*, **86:** 338–393.

von Holst, D. 2001. Social stress in wild mammals in their natural habitat. *In*: Broom, D. M. (Ed.). *Coping with challenge: Welfare in animals including humans*, pp 317–335. Berlin: Dahlem University Press.

von Holst, D., Hutzelmeyer, H., Kaetzke, P., Khaschei, M., Rödel, H. G. & Schrutka, H. 2002. Social rank, fecundity and lifetime reproductive success in wild European rabbits (*Oryctolagus cuniculus*). *Behavioral Ecology and Sociobiology*, **51**: 245–254.

Wallage-Drees, M. & Michielsen, N. C. 1989. The influence of food supply on the population dynamics of rabbits, *Oryctolagus cuniculus* (L.), in a Dutch dune area. *Zeitschrift für Säugetierkunde*, **54**: 304–323.

Wallage-Drees, M. 1989. A field study on seasonal changes in circadian activity of rabbits. *Zeitschrift für Säugetierkunde*, **51**: 26–36.

Winnie, J. & Creel, S. 2007. Sex-specific behavioural responses of elk to spatial and temporal variation in the threat of wolf predation. *Animal Behaviour*, **73**: 215–225.

CAPÍTULO 5

Medida no invasiva de la respuesta fisiológica de estrés de los conejos al olor de un depredador

Manuscrito original del artículo publicado en Chemoecology:

Monclús, R., Rödel, H. G., Palme, R., von Holst, D. & de Miguel, J. 2006. Non-invasive measurement of the physiological stress response of wild rabbits to the odour of a predator. Chemoecology, 16: 25-29.

Medida no invasiva de la respuesta fisiológica de estrés de los conejos al olor de un depredador

Resumen

Se han realizado muchos estudios sobre el estrés en diferentes mamíferos, pero la reacción fisiológica de estrés que el olor de un depredador puede inducir en las presas no ha recibido mucha atención. Además, no todos los animales responden igual a un agente estresante conocido.

Desarrollamos un procedimiento experimental con once conejos (*Oryctolagus cuniculus*) sin experiencia previa con depredadores, para determinar la respuesta fisiológica individual a la detección olfatoria de un depredador potencial. Los conejos se alojaron individualmente en pequeñas instalaciones con una madriguera artificial y con agua y comida disponible *ad libitum*. Los animales siguieron una fase control, sin olor, y una fase experimental, durante la que confrontamos a los conejos con el olor de zorros (*Vulpes vulpes*). Además, otra muestra de once conejos siguió un procedimiento control, mantenidos en las mismas condiciones de alojamiento y de manejo que los animales experimentales, pero sin encontrar olor de depredador. Para evaluar la respuesta fisiológica, analizamos la concentración de los metabolitos de los glucocorticoides en las heces de los conejos. Por lo tanto, cada día recogimos muestras de heces y mediante un inmunoensayo enzimático medimos los metabolitos de la corticosterona, en particular, aquellos metabolitos con una estructura 5α-3β, 11 β -diol.

Una vez que validamos el ensayo para los conejos, encontramos que la presencia simulada de un depredador (olor del zorro) en la instalación provocó un aumento de la concentración de metabolitos de la corticosterona en heces. Sin embargo, el agente estresante no afectó de la misma manera a todos los animales. Encontramos un aumento general en las diferencias individuales. En particular, los machos experimentaron un aumento mayor que las hembras, aunque la respuesta global fue similar en ambos sexos.

A nuestro entender, este es uno de los primeros intentos de analizar la evaluaciónd del riesgo de depredación por medio de métodos no invasivos.

Non-invasive measurement of the physiological stress response of wild rabbits to the odour of a predator

Summary

Stress has been widely studied in different mammals, but the physiological stress reaction that the odour of a predator could induce in preys has not received much attention. Besides, not all the animals would respond to the same extent to a known stressor.

We developed an experimental procedure with eleven naïve European rabbits (*Oryctolagus cuniculus*) in order to determine the individual physiological response to the olfactory detection of a potential predator. The rabbits were housed singly in small enclosures with a concrete burrow system and food and water were available *ad libitum*. The animals followed a control trial, without odour, and an experimental trial where we confronted the rabbits with fox (*Vulpes vulpes*) odour. Furthermore, another sample of eleven rabbits followed a control procedure subjected to the same housing and handling procedures but without facing the predator odour. In order to assess the physiological response we analysed the concentration of glucocorticoid metabolites in the faeces of the rabbits. Therefore, everyday faecal samples were collected and analysed with an enzyme immunoassay in order to measure the corticosterone metabolites (CM), particularly, those metabolites with a 5α-3β, 1β-diol structure.

After validating the assay for wild rabbits, we found that the simulated presence of a predator (fox odour) in the enclosure resulted in an increase in faecal CM concentrations. However, the stressor did not affect all the animals in the same way. We found a general increase in the individual differences. In particular, males experienced a higher increase than females, though the overall response was similar for both sexes.

To our knowledge, this is one of the first attempts to analyse the assessment of the risk of predation by means of non-invasive methods.

INTRODUCTION

Normally animals have to deal with predators during their lifetime and being unsuccessful in this task could mean the total loss of fitness. Therefore, preys have developed some adaptations at different levels (i.e. morphological, behavioural, physiological level) in order to decrease the risk of being preyed (Endler 1991; Lima 1998; Kats & Dill 1998). One of such mechanisms is the recognition of nearby predators by their scent. This allows the prey to avoid the so perilous direct encounters and therefore minimises the risk of being killed.

Generally, the assessment of a risk is translated into a modification of the behaviour of the animal. Behavioural responses of preys to the odour of predators have been widely studied in mammals (Hennessy & Owings 1978; Gorman 1984; Dickman & Doncaster 1984; Caine & Weldon 1989; Jedrzejewski & Jedrzejewska 1990; Ward *et al*. 1997; Burwash *et al*. 1998; Jonsson *et al*. 2000; Blumstein *et al*. 2002; Monclús *et al*. 2005). However, the recognition of a predator is not always associated with a behavioural response and this may lead to misinterpretations of the results attained (Ydenberg & Dill 1986). For instance, in those animals in which the costs of the behavioural response surpass the potential benefits, antipredator behaviours might not be present (Blumstein 2002). A complementary approach could be the measurement of the physiological stress response elicited by the recognition of the predator. Predator odour can be a strong stressor, which should activate the hypothalamic pituitary adrenocortical axis (hereafter HPA) and the sympatheticoadrenomedullary system (von Holst 1998; Matteri *et al.* 2001; Möstl & Palme 2002). This would lead to an increase of glucocorticoid and catecholamine levels in the blood, respectively, which are responsible for mobilization of energy. Nevertheless, individual differences between animals in the stress response have been highlighted in several studies (e.g. Cockrem & Silverin 2002).

There are different approaches to the measurement of glucocorticoids. Invasive methods such as blood sampling require trapping, handling and puncture. However, all these procedures affect the glucocorticoid concentrations in blood within a few minutes. Furthermore, especially in small mammals, serial bleeding is not viable (von Holst 1998; Touma & Palme 2005). On the other hand, the use of non-invasive techniques, such as the

analysis of glucocorticoid metabolites in the faeces, is highly desirable, as faecal samples can be collected easily without disturbing the animal. Furthermore, since serial sampling is feasible, it could provide information about the individual variation among animals. The technique has been established and validated in several species and the suitability for its use in wild animals has been confirmed (reviewed in Möstl & Palme 2002; Touma & Palme 2005).

The aim of the study was to analyse the individual physiological stress response in adult European rabbits (*Oryctolagus cuniculus*) to the odour of a predator. Apart from differences among individuals, we expected to find sexual differences due to different baseline levels or different metabolic routes (Palme *et al*. 1996; Schatz & Palme 2001; Touma *et al*. 2003). Besides, we had to validate this non-invasive technique for wild rabbits since the assay was firstly developed in mice (Palme 2005; Touma & Palme 2005).

METHODS

Animals and housing conditions

The animal experiment was carried out at the Department of Animal Physiology of the University of Bayreuth, Germany. All animals were cared for in accordance with institutional guidelines, and the experiments were announced to the responsible authorities (government of Middle Franconia, Germany, 621-2531.32-1/04). We used eleven (six females and five males) adult European rabbits, which were about eight months old. Another group of eleven animals (two males and nine females) which were 7-8 months old were used to control for the possible responses due to handling and housing conditions (see below for details). All animals were descendants from wild individuals that had been caught at different sites in south Germany in 1984. The animals were raised in social groups in a field enclosure where mammalian predators were excluded by means of two electric fences. During the experiments, the individuals were housed individually in outdoor wire mesh enclosures with sandy soil. Digging was inhibited by a wire mesh layer underneath the sand, and on the top of the enclosure a wire mesh was used to exclude raptors. Each of these enclosures (360 x 460 cm) contained an artificial concrete burrow consisting of a tube

(length: 150 cm, diameter: 20 cm) and a chamber with a removable top (diameter: 60 cm). In total, six of these enclosures were available for the experiments. To assure that rabbits detected the odours, the scents were placed next to the food. There were two wooden feeding boxes (30 x 30 x 30 cm) per enclosure. One of the sides was left open to allow the rabbits to enter. Each box contained two feeding dishes. In the inner bowl we placed the odour and in the outer the food pellets. Within each enclosure, the feeding boxes were 3 m apart from each other and from the burrow, forming an equilateral triangle. Water was provided *ad libitum* and everyday we placed in each box 75 g of rabbit food pellets, which exceeds the daily food requirements of the rabbit (cf. Bini & Xiccato 1998).

Experimental design

Rabbits were left to acclimatise for 20 days within the enclosures. After the habituation period they followed two consecutive trials. The first trial aimed on evaluating basal levels of adrenocortical activity, whereas the aim of the second trial was to evaluate the response to a known stressor, the presence of a simulated predator. Therefore, during the first trial (hereafter non-fox odour trial) no odours were presented but during the second trial (hereafter foxodour trial) fresh fox faeces were placed at random in one of the two feeding boxes. Due to possible preferences for one of the boxes, the trial was repeated, we shifted the boxes and we averaged the values registered in both trials. In summary, rabbits were subjected to a two-choice experiment, so even if the odour of the fox provoked aversion in the rabbits, they had a further option to feed. The total length of the experiment was 14 days. The non-fox odour trial lasted 9 days, in order to have a reliable measure of the basal levels. The fox-odour trial lasted 5 days.

In order to exclude possible additive effects of the trials, which could result in an increase of the glucocorticoid levels at the end of the experiment, we performed a control with another 11 rabbits. They were housed and handled similarly to the experimental animals but they were not exposed to a predator odour. We expected that rabbits would not show an increase in glucocorticoid metabolites during this procedure in contrast to the experimental animals.

Predator odours

All the fox excrements were collected from captive animals of Hof Zoological Park (Franconia, Germany). The faeces were wrapped individually in aluminium foil and were frozen at –20 ºC until shortly before the experiment took place.

Collection and analysis of rabbit faeces

Rabbit pellets were collected daily in the morning during the whole procedure. The sampling was done only once a day in order to prevent further disturbance. As rabbits were housed individually, all the faeces within one enclosure belonged to the resident. The rest of the faeces of that enclosure were removed in order to ensure that the samples collected everyday were excreted during the night before. Immediately after collection, the samples were frozen at –60º C.

We collected the faeces approximately 12 h after the rabbits encountered the odour for the first time, as it is the average time until the excretion peak is registered in rabbits (Piekarz 1963). Due to the high variability expected in the time course of the excretion (Piekarz 1963), we averaged the obtained concentrations of CM per animal and per trial. Each sample was homogenised with mortar and pestle and 0.2 g of each was weighed with the help of a precision balance. The volume of the sample was taken up to 0.5 ml by means of adding water. The metabolites were suspended with 5 ml of methanol (80 %) as described before (Palme and Möstl 1997; Teskey-Gerstl *et al*. 2000). After vortexing for 30 minutes, the samples were centrifuged and a dilution (1:10 with assay buffer) of the supernatant was transferred into a new vial and frozen until analysis. For the analysis of CM in the faeces we used an already established enzyme immunoassay (EIA), measuring metabolites with a 5α-3β, 11β-diol structure. This EIA was developed for laboratory mice *Mus domesticus* (for details see Touma *et al.* 2003; 2004).

Physiological validation of the assay

We validated the assay by means of an ACTH challenge test (Touma & Palme 2005). Rabbits (n_{total} = 16; 10 females and 6 males) were injected intramuscularly with 0.1 g of synthetic ACTH (Synacthen, Novartis, Germany). We used 5 animals from the experimental group and 11 animals from the control group. The validation was done at the

beginning of the experiment. Faeces were collected before and on the two following days after ACTH injection (see Fig. 1).

Statistical analysis

For all parametric statistical tests, we ensured that the variables were normally distributed (Shapiro Wilk test) and that variances were homogenous (Levene test). In order to assess the individual variability in the response to a stressor, we calculated the coefficients of variation between animals. For that purpose, we used the mean of all the values measured of each animal in each trial.

RESULTS

We could positively validate the assay (repeated-measures ANOVA: $F_{1,15} = 26.529$, $P < 0.001$; Fig. 1). After the injection of ACTH, the values of the faecal CM increased by 174% (paired t-test: $t_{15} = -5.140$; $P < 0.001$). We did not find significant differences in the initial increase experienced by males and females (t-test: $t_{14} = 1.555$, $P = 0.173$).

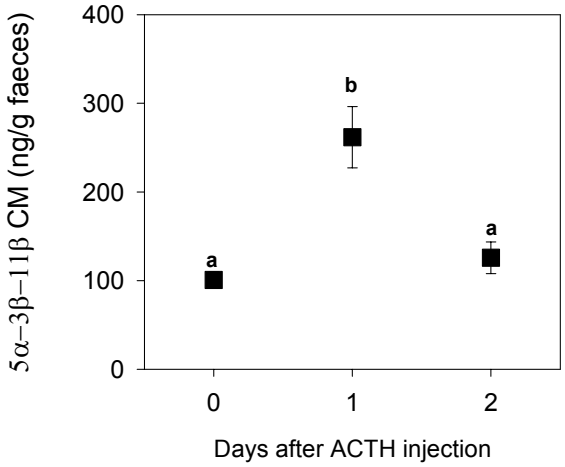

Figure 1. Concentrations of faecal corticosterone metabolites (5α-3β,11β-diol CM; mean ±SE) after an injection of synthetic ACTH (Synacthen, 0.1 g/ml). Different letters indicate significant ($P < 0.050$) differences according to paired *t*-tests post hoc to a repeated measures ANOVA (see text for statistics).

The faecal CM concentrations of the animals of the control group and the experimental group did not differ significantly during the non-fox odour trial (two-way ANOVA: $F_{1,18} = 1.180$, $P = 0.292$). We also did not find statistically significant differences in these initial values between individuals of both sexes ($F_{1,18} = 3.202$, $P = 0.090$). However, the CM values of the males tended to be higher, on average 39.3% (males: 94.3 ng/g faeces ± 24.0 SD; females: 67.8 ng/g faeces ± 18.7 SD). The interaction between both factors was not significant (group × sex: $F_{1,18} = 1.404$, $P = 0.251$).

After confronting the animals of the experimental group with fox faeces next to one of their two feeding bowls, we detected a strong increase (% change) in the faecal CM concentration. This change differed significantly from the one observed in the control group (t-test: $t_{20} = -3.034$; $P = 0.006$; Fig. 2), whereas the males that were exposed to predator odour displayed significantly higher values than females (47.68% higher) ($Z = -2.646$; $n_{\text{males}} = 4$; $n_{\text{females}} = 7$; $P = 0.008$). The average values of males and females, respectively, were 126.62 ± 25 SD and 68.41 ± 17.6 SD. Nevertheless, these sex differences were not apparent any more with respect to the percentage of change in the experimental group (t-test: $t_9 = -1.040$; $P = 0.330$).

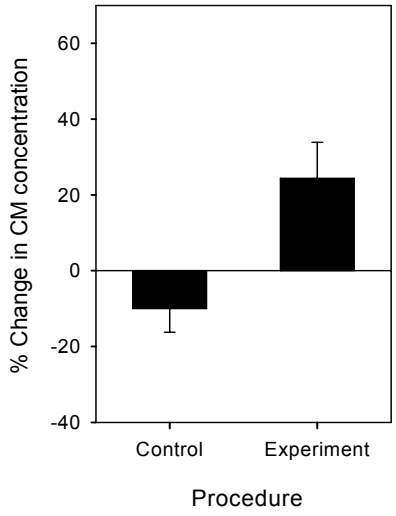

Figure 2 Comparison of the percentage of change in the faecal CM concentrations between the control group and the experimental group. In the experimental group ($n_{\text{individuals}} = 11$) fox odour was presented during the second trial but not during the first trial. In the control group ($n_{\text{individuals}} = 11$) no fox odour was presented during both trials. See text for statistics.

We found that during the fox-odour trial two of the animals did not show any increase in their metabolites. However all the other animals showed a notable increase (> 10%). In five of these animals, the CM concentrations increased 12 hours after encountering the odour, and in the other four, the increase was apparent 24 h later (36 h since the first encounter). These differences registered in the time course of excretion (12h or 36h) were not due to the sex of the animals (Fisher's Exact: $P = 0.524$).

The coefficients of variation (CV) for the experimental group were 29.0% during the non-fox odour trial and increased to 39.2%, when fox odour was presented. However, for the control animals the values did not increase between trials, being the CV 27.9% and 24.7%, respectively.

DISCUSSION

The measurement of glucocorticoid metabolites in the faeces has been shown to be a useful tool for registering the assessment of the risk of predation in European rabbits. Our results proved that the rabbits were able to recognise a predator by means of its odour and, as a consequence, they exhibited a physiological stress response. However, not all the animals responded to the same extent. We found sex differences when subjected to a stressful situation as well as inter-individual differences in the occurrence of the excretion peaks.

By means of our double validation (ACTH challenge test and the experiment itself) and our standardised set up we could prove that the EIA we used, which was firstly established in mice (Touma *et al*. 2003), is adequate for its use in wild rabbits.

With our experimental set up we could corroborate the results attained before (Monclús *et al*. 2005). When encountering the predator odour, the rabbits experienced an increase in their CM, which could be assigned to their assessment of the risk of predation. Under stress, the activation of the HPA axis, together with the activation of the sympathetico-adrenomedullary system, contributes to the mobilization of energy necessary to cope with the stressful situation (Boissy 1995; von Holst 1998; Holberton & Able 2000; Buchanan 2000; Creel 2001). In spite of the importance of this mechanism in the stress response, and given the fact that behavioural responses are not always displayed (Blumstein

2002; but see Calder & Gorman 1991), there are only a few studies focusing on the physiological responses to the odour of a predator (Vernet-Maury *et al*. 1984; Lima 1998). However, in any situation one would expect to find a physiological response, as different stressors lead to the activation of the same axis. It has been shown that in species where the rate of encounter with predators is low, animals display a physiological response, which is not translated into a modification of behaviour (Eilam *et al*. 1999).

The measurement of the physiological response by means of non-invasive methods, allowed us to take many samples from each individual so we could assess the high degree of individual variation in the release of corticosterone in response to a uniform stressor. Cockrem & Silverin (2002) found that in great tits (*Parus major*), the increase of plasma corticosterone levels after handling differed between the birds, but was quite conservative within individuals. It is well known that animals cope with stressors in different ways, which could explain the variability observed (Benus *et al*. 1987; Sapolsky 1990; Wingfield *et al*. 1995; Campbell *et al*. 2003). One possible factor affecting those differences could be the sex of the animals (Warner 1981). In our study, males showed higher values than females. In contrast, Boonstra and co-workers (2001) found in arctic ground squirrels (*Spermophilus parryii*) a stronger reaction in females than in males.

Sexual differences in the physiological stress response have been reported in other species (Touma and Palme 2005) and they are supposed to be partly due to differences in the metabolism of glucocorticoids in males and females (Palme *et al*. 1996; Schatz & Palme 2001; Touma *et al*. 2003). Accordingly, we (Monclús *et al*. in press) did not find differences between males and females in the corticosterone challenge values in serum, supporting the idea of the differential metabolic pathways. However, even if the males had higher values, both males and females showed a similar reaction to the stressor, which could indicate that the differences registered in the absolute values were mainly due to differences in the initial levels.

As expected, the rabbits showed a high variability in the time course of faecal excretion. Piekarz (1963) found that in domestic rabbits, which were mainly nocturnal, as in our case (9 out of 11 were exclusively nocturnal), peak excretion occurred on average 12 h after ingestion. However, due to the high individual variability, they oscillated from 5 to 20 h. The fact that some of the rabbits experienced an increase in the CM later than expected

could suggest that the differences were due to the variability in the excretion peaks. Moreover, there are several factors that influence retention times, e.g. coprophagy, age, activity, pregnancy, ambient temperature (Piekarz 1963, Warner 1981). As the measurement of faeces CM strongly relies on the time course of excretion, all these factors should be taken into account.

To our knowledge, this is one of the first attempts to trace the physiological stress response elicited by the odour of a predator by means of non-invasive techniques. The measurement of faecal glucocorticoid metabolites is becoming an essential tool in many disciplines, such as animal welfare and conservation biology as it avoids trapping and handling. EIA's have been validated in many different species (reviewed in Möstl & Palme 2002; Möstl *et al*. 2005; Palme 2005, Touma & Palme 2005). However there are still some issues which need improvement, specially when working with free ranging species where sex, rank and reproductive status cannot be achieved so easily (Huber *et al*. 2003; Touma & Palme 2005). Further research should be done in order to study the responses in more complex situations, where social interactions and different trade-offs could modulate the responses.

Acknowledgements

We are especially grateful to Andreas Zechner who gave us essential technical support. The manuscript was improved by helpful comments from Sophie Rettenbacher, Dan Blumstein and an anonymous reviewer. R. M. was supported by an FPI scholarship from the Comunidad Autónoma de Madrid, Spain.

REFERENCES

Benus, R. F., Koolhaas, J. M. & van Oortmerssen, G. A. 1987. Individual differences in behavioural reaction to a changing environment in mice and rats. *Behaviour*, **100**: 105–122.

Bini, R. P. & Xiccato, G. 1998. Energy metabolism and requirements. *In*: de Blas, C. & Wieseman, J. (Eds.). *The nutrition of the rabbit*, pp: 103-122. New York: CABI Publishing.

Blumstein, D. T. 2002. Moving to suburbia: ontogenetic and evolutionary consequences of life on predator-free islands. *Journal of Biogeography*, **29**: 685–692.

Blumstein, D. T., Mari, M., Daniel, J. C., Ardron, J. G., Griffin, A. S. & Evans, C. S. 2002. Olfactory predator recognition: wallabies may have to learn to be wary. *Animal Conservation*, **5**: 87–93.

Boissy, A. 1995. Fear and fearfulness in animals. *The Quarterly Review of Biology*, **70**: 165–191.

Boonstra, R., Hubbs, A. H., Lacey, E. A. & McColl, C. J. 2001. Seasonal changes in glucocorticoid and testosterone concentrations in free-living arctic ground squirrels from the boreal forest of the Yukon. *Canadian Journal of Zoology*, **79**: 49–58.

Buchanan, K. L. 2000. Stress and the evolution of condition-dependent signals. *Trends in Ecology and Evolution*, **15**: 156–160.

Burwash, M. D., Tobin, M. E., Woolhouse, A. D. & Sullivan, T. P. 1998. Laboratory evaluation of predator odors for eliciting an avoidance response in roof rats (*Rattus rattus*). *Journal of Chemical Ecology*, **24**: 49–66.

Caine, N. G. & Weldon, P. J. 1989. Responses by red-bellied tamarins (*Saguinus labiatus*) to fecal scents of predatory and nonpredatory neotropical mammals. *Biotropica*, **21**: 186–189.

Calder, C. J. & Gorman, M. L. 1991. The effects of red fox *Vulpes vulpes* faecal odours on the feeding behaviour of Orkney voles *Microtus arvalis*. *Journal of Zoology, London*, **224**: 599–606.

Campbell, T., Lin, S., DeVries, C. & Lambert, K. 2003. Coping strategies in male and female rats exposed to multiple stressors. *Physiology and Behavior*, **78**: 495–504.

Cockrem, J. F. & Silverin, B. 2002. Variation within and between birds in corticosterone responses of great tits (*Parus major*). *General and Comparative Endocrinology*, **125**: 197–206.

Creel, S. 2001. Social dominance and stress hormones. *Trends in Ecology and Evolution*, **16**: 491–497.

Dickman, C. R. & Doncaster, C. P. 1984. Responses of small mammals to red fox (*Vulpes vulpes*) odour. *Journal of Zoology, London*, **204**: 521–531.

Eilam, D., Dayan, T., Ben-Eliyahu, S., Schulman, I., Shefer, G. & Hendrie, C. A. 1999. Differential behavioural and hormonal responses of voles and spiny mice to owl calls. *Animal Behaviour*, **58**: 1085–1093.

Endler, J. A. 1991. Interaction between predators and prey. *In*: Krebs, J. R. & Davies, N. B. (Eds). *Behavioural Ecology – An Evolutionary Approach. 3rd Ed.*, pp 169–196 Oxford: Blackwell Science.

Gorman, M. L. 1984. The response of prey to stoat (*Mustela erminea*) scent. *Journal of Zoology, London*, **202**: 419–423.

Hennessy, D. F. & Owings, D. H. 1978. Snake species discrimination and the role of olfactory cues in the snake-directed behaviour of the California ground squirrel. *Behaviour*, **65**: 115–124.

Holberton, R. L. & Able, P. A. 2000. Differential migration and an endocrine response to stress in wintering dark-eyed juncos (*Junco hyemalis*). *Proceedings of the Royal Society of London B*, **267**: 1889–1896.

Huber, S., Palme, R. & Arnold, W. 2003. Effects of season, sex, and sample collection on concentrations of fecal cortisol metabolites in red deer (*Cervus elaphus*). *General and Comparative Endocrinology*, **130**: 48–54.

Jedrzejewski, W. & Jedrzejewska, B. 1990. Effect of a predator's visit on the spatial distribution of bank voles: experiments with weasels. *Canadian Journal of Zoology*, **68**: 660–666.

Jonsson, P., Koskela, E. & Mappes, T. 2000. Does risk of predation by mammalian predators affect the spacing behaviour of rodents? Two large-scale experiments. *Oecologia*, **122**: 487–492.

Kats, L. B. & Dill, L. M. 1998. The scent of death: chemosensory assessment of predation risk by prey animal. *Ecoscience*, **5**: 361–394.

Lima, S. L. 1998. Stress and decision making under the risk of predation: Recent developments from behavioral, reproductive and ecological perspectives. *Advances in the Study of Behavior*, **27**: 215–290.

Matteri, R. L., Carroll, J. A. & Dyer, C. J. 2001. Neuroendocrine responses to stress *In*: Moberg, G. P. & Mench, J. A. (Eds.). *The biology of animal stress. Basic principles and implications for animal welfare*, pp 43–76. CABI Publishing.

Monclús, R., Rödel, H. G., von Holst, D. & Miguel, F. J. 2005. Behavioural and physiological responses of naïve European rabbits to predator odour. *Animal Behaviour*, **70**: 753–761.

Möstl, E. & Palme, R. 2002. Hormones as indicators of stress. *Domestic Animal Endocrinology*, **23**: 67–74.

Möstl, E., Rettenbacher, S. & Palme, R. 2005. Measurement of corticosterone metabolites in birds' droppings: An analytical approach. *Annals of the New York Academy of Science*, **1046**: 17–34.

Palme, R. 2005. Measuring fecal steroids: guidelines for practical application. *Annals of the New York Academy of Science*, **1046**: 75–80.

Palme, R., Fischer, P., Schildorfer, H. & Ismail, M. N. 1996. Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock. *Animal Reproduction Science*, **43**: 43–63.

Palme, R. & Möstl, E. 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *International Journal of Mammalian Biology Suppl. II*, **62**: 192–197.

Piekarz, R. 1963. The influence of coprophagy on the time of passing of food down the alimentary tract in the domestic rabbit. *Acta Physiologica Polonica* **14**: 337–348.

Sapolsky, R. M. 1990. Stress in the wild. *Scientific American*, **262**: 116–123.

Schatz, S. & Palme, R. 2001. Measurement of faecal cortisol metabolites in cats and dogs: a non-invasive meted for evaluating adrenocortical function. *Veterinary Research Communications*, **25**: 271–287.

Teskey-Gerstl, A., Bamberg, E., Steineck, T. & Palme, R. 2000. Excretion of corticosteroids in urine and faeces of hares (*Lepus europaeus*). *Journal of Comparative Physiology B*, **170**: 163–168.

Touma, C., Sachser, N., Möstl, E. & Palme, R. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *General and Comparative Endocrinology*, **130**: 267–278.

Touma, C., Palme, R. & Sachser, N. 2004. Analyzing corticosterone metabolites in fecal samples of mice: A noninvasive technique to monitor stress hormones. *Hormones and Behavior*, **45**: 10–22.

Touma, C. & Palme, R. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of a validation. *Annals of the New York Academy of Science*, **1046**: 54–74.

Vernet-Maury, E., Polak, E. H. & Demael, A. 1984. Structure-activity relationship of stress-inducing odorants in the rat. *Journal of Chemical Ecology*, **10**: 1007–1018.

von Holst, D. 1998. The concept of stress and its relevance for animal behavior. *Advances in the Study of Behavior*, **27**: 1–131.

Ward, J. F., Macdonald, D. W. & Doncaster, C. P. 1997. Responses of foraging hedgehogs to badger odour. *Animal Behaviour*, **53**: 709–720.

Warner, A. C. I. 1981. Rate of passage of digesta through the gut of mammals and birds. *Nutrition Abstracts and Reviews, Series B*, **51**: 789–820.

Wingfield, J. C., O'Reilly, K. M. & Ashteimer, L. B. 1995. Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. *American Zoologist*, **35**: 285–294.

Ydenberg, R. C. & Dill, L. M. 1986. The economics of fleeing from predators. *Advances in the Study of Behavior*, **16**: 229–249.

CAPÍTULO 6

Posibles aplicaciones de los resultados

Estilos comportamentales en el conejo: las interacciones sociales y las respuestas a agentes estresantes experimentales

Manuscrito original del artículo publicado en Physiology & Behavior:

Rödel, H. G., Monclús, R. & von Holst, D. 2006. Behavioral styles in European rabbits: social interactions and responses to experimental stressors. Physiology & Behavior, 89: 180-188.

Los resultados obtenidos pueden ser útiles en el manejo y en la gestión del conejo. El análisis detallado de las respuestas de los conejos al olor de depredadores sugiere que la utilización de los olores de los depredadores probablemente no sea una medida efectiva en el control de plagas en condiciones naturales, ya que las respuestas de evitación que los conejos desarrollaron en el laboratorio, no se reprodujeron en semilibertad. Además, el olor del depredador no produjo cambios en el uso del espacio, lo que indica el valor del territorio para los conejos. Asimismo se ha puesto de manifiesto la importancia del grupo social. Los animales, en particular las hembras, en grupos inestables dedican gran parte de su tiempo a las conductas agonísticas, tiempo que no dedican a otras actividades con más relevancia para su éxito biológico, tales como evitar depredadores. Estos hechos deberían tenerse en cuenta a la hora de realizar reintroducciones o traslados de ejemplares.

Por otro lado, una posible aplicación podría estar relacionada con los experimentos en los que se requiere un agente estresante, como por ejemplo, aquéllos en los que se pretende estudiar las respuestas fisiológicas de estrés de una especie o los estilos comportamentales. En muchos de estos experimentos los animales se someten a condiciones a las que en condiciones naturales rara vez se enfrentarían, como la inmovilidad, el transporte, o incluso las descargas eléctricas. Sería deseable que las condiciones a las que se enfrentan los animales en el laboratorio tuvieran relación con la biología de la especie y con los retos a los que se enfrentan en su medio natural. De no ser así, las conclusiones a las que pueden conducir estos experimentos no tendrán un significado biológico, y en la mayor parte de los casos el resultado será un artefacto de laboratorio. Los animales en condiciones naturales se enfrentan a diario a sus depredadores. Hemos comprobado que el reconocimiento de los depredadores por el olor constituye un agente estresante para los conejos, que modifica su comportamiento y desencadena en el laboratorio una respuesta fisiológica de estrés. Por lo tanto, en el trabajo que se presenta a continuación empleamos la presencia de un depredador como un agente estresante más.

Estilos comportamentales en el conejo: las interacciones sociales y las respuestas a agentes estresantes experimentales

Resumen

La existencia y consistencia de los tipos de comportamiento individuales en respuesta a las situaciones que suponen un reto es de interés creciente en biología del comportamiento. En nuestro estudio con conejos (*Oryctolagus cuniculus*), (1) investigamos las correlaciones entre el comportamiento social durante el desarrollo temprano y las respuestas a agentes estresantes experimentales en etapas posteriores, y (2) comprobamos las consistencias en estas respuestas en diferentes situaciones. Para ello, observamos a los juveniles que viven en una instalación natural al principio del verano y registramos las interacciones agonísticas y sociopositivas. En otoño, los animales se (a) introdujeron en solitario en un ambiente nuevo y (b) se confrontaron con olor de depredador (zorro, *Vulpes vulpes*). Registramos las respuestas comportamentales y fisiológicas de estrés. Además, evaluamos la prueba del olor del depredador con una muestra independiente de animales. Estos últimos resultados mostraron una correlación entre el comportamiento y la respuesta fisiológica de los animales. Los individuos que reaccionaron a la presencia del olor de zorro por medio de una tasa baja de vigilancia, mostraron un gran aumento de corticosterona en suero, mientras que los niveles de los que aumentaron mucho la vigilancia permanecieron estables. En general, encontramos correlaciones entre el comportamiento social exhibido durante el desarrollo temprano y las respuestas comportamentales en dos pruebas experimentales. Sin embargo, la correlación entre las diferentes características del comportamiento social y las respuestas durante las dos pruebas experimentales no fueron consistentes. Los animales que participaron en más interacciones agonísticas durante su desarrollo temprano, empezaron antes a explorar cuando se les introdujo en el ambiente nuevo. Durante la segunda prueba encontramos que los conejos que previamente habían mostrado una frecuencia mayor de comportamiento social positivo respondieron a la presencia de olor de depredador vigilando más. Además, las respuestas comportamentales durante ambas pruebas experimentales no se correlacionaron: los que exploraban más en la prueba del ambiente nuevo no mostraron una respuesta más activa en la prueba del olor de depredador. Debido a la falta de estilos de comportamiento consistentes en ambas

pruebas, concluimos que el estudio no apoya la existencia de fenotipos comportamentales de ámbito general en los conejos.

Behavioral styles in European rabbits: Social interactions and responses to experimental stressors

Abstract

The existence and consistency of individual behavioral types in response to challenging situations is of increasing interest in behavioral biology. In our study on European rabbits (Oryctolagus cuniculus), we (1) investigated correlations between social behavior during early development and responses to experimental stressors during later life, and (2) tested for consistencies in these responses across different situations. For this, we observed juveniles living in field enclosures in early summer and recorded agonistic and positive social interactions. In autumn, the animals were (a) introduced singly into a novel environment and were (b) confronted with predator (red fox *Vulpes vulpes*) odor. We recorded behavioral and physiological stress responses. In addition, we evaluated the predator odor test with an independent sample of animals. These latter results showed a correlation between the animals' behavioral and physiological response: Individuals, which reacted to the presence of fox odor by low scanning rates showed a high increase in serum corticosterone challenge concentrations, whilst the levels in high scanners remained stable. Overall, we found correlations among social behavior displayed during early development and behavioral responses in the two experimental tests, however the correlations between the different traits of social behavior and the responses during the two different experimental tests were not consistent. Animals which were involved in more agonistic interactions during their early development started to explore faster when entered into the novel environment. During the second test we found that rabbits which previously showed a higher frequency of positive social behavior responded to the presence of predator odor by more scanning. Moreover, the behavioral responses during both experimental tests were not correlated: fast explorers in the novel environment test did not show a more active response during the predator odor test. Due to this lack of consistent behavioral styles across both tests we conclude that the study fails to support the existence of domain-general behavioral phenotypes in European rabbits.

1. INTRODUCTION

The behavioral responses to a given stimulus often vary considerably among individuals (Wilson 1998; Gosling 2001; Sih *et al.*2004). Studies on many animal species differentiate two principal behavioral styles reflecting how an individual can deal with a stressful or challenging situation. With respect to animal temperament, individuals can be classified as bold or shy (Kagan *et al.* 1988; Suomi 1991;Verbeek *et al.* 1994; Wilson *et al.* 1994; Clarke & Boinski 1995). According to another conceptual framework, the behavioral and also physiological effort to master a stressful situation is commonly referred to as coping style (Henry & Stephens 1977; Benus *et al.* 1991; von Holst 1998; Koolhaas *et al.* 1999; Koolhaas *et al.* 2001). Even if such categorizations might only reflect the extremes of behavioral continua, there is convincing evidence that such different behavioral styles exist, for example in dogs (*Canis familiaris*), rodents, domestic pigs (*Sus scrofa*), great tits (*Parus major*) and tree shrews (*Tupaia belangeri*) (Corson & Corson 1976; von Holst 1986; Benus *et al.* 1987; Hessing *et al.* 1994; Campbell *et al.* 1996; Verbeek *et al.* 1996; Sih *et al.* 2004). Generally, individuals with a more active coping style will show a tendency to actively manipulate their current stressful situation while passive individuals will not (Henry & Stephens 1977; von Holst 1998). A similar classification suggests a differentiation between proactive (i.e. more active) individuals and reactive ones, whereas the latter will try to adjust themselves to their current situation but will tend to show a higher flexibility in their responses (Koolhaas *et al.* 1999). Or, with reference to the classification on animal temperament, shy individuals will show avoidance while bold animals will demonstrate interest when confronted with a novel or challenging situation. These behavioral styles are often found to occur consistently over the time and also across a variety of different experimental situations (i.e. under different given sets of conditions) (Koolhaas *et al.* 1999, Sih *et al.* 2004). At the individual level, distinctive patterns of behavior may be used to describe the behavioral phenotype or personality of an animal (Pervin & John 1997; Gosling 2001; Carere *et al.* 2005). Furthermore, several studies point out that the early development of an individual might play an important role in modulating the behavioral patterns of an

individual during its later life (Sachser 1986; Anisman *et al.* 1998; Meerlo *et al.* 1999; Kaiser 2003; Macrí *et al.* 2004).

Several studies have addressed the question of the existence of distinct behavioral phenotypes in feral or wild bird and mammal populations, for example in spotted hyenas (*Crocuta crocuta*), bighorn sheep (*Ovis canadensis*) and extensive studies in great tits (*P. major*) (Gosling 1998; Réale *et al.* 2000; Dingemanse *et al.* 2002; Groothuis & Carere 2005). However, only few data are available which link the individual social behavior of animals living in their natural environment with behavioral and physiological stress responses to experimental stressors (Armitage 1986; Armitage & van Vuren 2003). We conducted such a study on juvenile European rabbits (*Oryctolagus cuniculus*, descendants of wild animals) living under semi-natural conditions. European rabbits are organized in social groups. The young rabbits leave the breeding burrow for the first time at the age of 20 days and start to interact with adults and other juveniles (Lockley 1961; Cowan 1987).

In our experiments, we confronted the animals with two different stressors. The first challenge was a novel environment test. It is known that animals which have the general tendency to act in a more (pro)active way start to explore their environment faster than more passive individuals (Huntingford 1976; Verbeek *et al.* 1994; Verbeek *et al.* 1996; Carere *et al.* 2005). We secondly exposed the animals to fox (*Vulpes vulpes*) odor. The presence of a potential predator also represents a strong and naturalistic stressor to which animals may react by displaying a variety of different behavioral and/or physiological responses. Many mammal species are capable of assessing this potential threat by olfactory cues, e.g. by the odor of a mammalian predator's urine or feces, where the ability to detect might be genetically fixed or learnt (Kats & Dill 1998; Lima 1998; Apfelbach *et al.* 2005: Takahashi *et al.* 2005). Our previous studies revealed that in European rabbits, the detection ability of the presence of fox odor is independent of prior experience. Under the standardized conditions of an anti-predator-response test, the animals generally reacted by increasing their vigilance and the responsiveness of their adrenocortical system (determined by their serum corticosterone concentrations after ACTH challenge). However, a high intra-individual variation in these responses was apparent (Monclús *et al.* 2005; Monclús *et al.* 2006). Such variation might be based on differences in the way how an animal copes with a given, challenging situation. Several studies on mammals and birds found that animals

which could be characterized as shy, slow (referring to their speed of exploration) and neophobic, display a higher HPA reactivity than bold, fast and neophilic individuals (Koolhaas *et al.* 1999; Cavigelli & McClintock 2003; Carere *et al.* 2003; Veenema *et al.* 2003).

The major goals of our study were (1) to investigate whether the social behavior during early life predicted the individual responses of European rabbits (a) when entered into a novel environment and (b) in response to the presentation of predator odor. We (2) also studied whether these responses occurred consistently over both test situations. If behavioral styles are consistent, we would expect that the same traits of social behavior during the early juvenile development would predict active response patterns during both test situations. Furthermore, more active responders during the novel environment test should also show a more active behavioral style during the subsequent predator odor test. Prior to the execution of these tests, we evaluated the intra-individual relationship between behavioral and physiological stress response to predator odor with an independent sample of animals. By the aid of this additional experiment, we tested our expectation that animals which actively controlled their current situation by higher scanning rates should show a lower increase in their glucocorticoid levels (e.g. von Holst 1986).

2. METHODS

2.1. Study animals

The study was conducted on European rabbits which were descendants of wild animals caught in South Germany in 1984. All focal animals were born and grew up in the semi-natural environment of two outdoor enclosures of 0.3 and 2.0 ha in size. Both enclosures were situated near the University of Bayreuth (Franconia, Germany). Vegetation in both enclosures consisted of homogeneous grassland. The population in the small enclosure was established about eight months (in autumn 2003) before the study started. The population in the two hectare sized enclosure was established in 1986. In both enclosures, the adult population density was similar during the study period (about 26.5 adults/ha during the

breeding season). Like in most European rabbit populations in the temperate zones, the juveniles did not actively take part in the reproductive process within their year of birth.

Both enclosures contained artificial burrow systems (big enclosure: 16 warrens; small enclosure: 3 warrens) with interconnected chambers and removable tops. However, the rabbits were not prevented from digging their own burrows. These natural burrows were prepared by us in such a way that checking for newborn litters was possible through artificial holes that we covered with flagstones.On the 12th day after birth, the nestlings were sexed, weighed and marked individually with colored plastic tags in both ears (Dalton Rototag: 20×5×1 mm3). Adult animals were also marked individually by plastic ear tags (Dalton Rototag: 35×10×2 mm3) and by a colored aluminum tag. Both enclosures were protected against the access of mammalian predators by a double electric fence.

2.2. Behavioral observations in semi-natural environment

European rabbits leave their breeding burrow for the first time at around day 20 after birth. We collected behavioral data from the 3rd to the 10th week after first emergence. The animals were observed for 30 min a day during the last 2–3 h before dusk. These observation units were equally distributed over the whole observation period resulting in an average observation time of 6.1 h (±1.7 S.E.) per juvenile. In total, we managed to collect data from 41 individuals (24 females, 17 males, Table 1). We noted down the frequencies of agonistic interactions with conspecifics (continuous recording) and, as a measure of positive social interactions, the time spent in close proximity to adults and other juveniles (one-zero sampling in 2 min intervals) (Martin & Bateson 1986). All behavioral variables were standardized per hour observation time.

For all focal animals that we later used for the reaction tests (n = 14, see below), we considered three behavioral variables:

Agonistic behavior (covariable): The total frequency of offensive (chasing, displacing) and defensive interactions (being chased, being displaced) with all other animals (adults+juveniles) of their social group. This variable can be considered as a measure of the juvenile's activity.

Offensive behavior (factor with two levels): As a measure of aggressiveness, we used the occurrence of offensive behavior (chasing, displacing) against other

juveniles. In many juveniles (see results section), we did not observe any display of offensive behavior during our recording sessions, indicating that in these animals the occurrence of this behavior was very low. Therefore, we categorized the frequency of offensive behavior in high and low by doing a median cut. Note that offensive behavior of juveniles against adults did not occur.

Positive social behavior (covariable): The proportion of observation time that the focal animal spent resting or feeding in close proximity (≤juvenile body length) to other animals (adults+juveniles). Staying in close proximity to a conspecific is a commonly used index for describing a positive social relation among interaction partners (Gust *et al.* 1996; Hennessy 1997; Barrett & Henzi 2002).

By using these variables, we tested for relationships between social behavior during the early juvenile phase and responses during the two test procedures.

Table 1. Timetable of the study

Experimental period	Time of year	Sample size
Behavioral observations in semi-natural environment	Spring-Summer	$n = 41$
Test 1: Evaluation of the predator odor test	Autumn	$n = 25$
Test 2: Novel environment test	Autumn	$n = 14$
Test 3: Predator odor test	Autumn	$n = 14$

Table 1. Note that we only observed a sample (n = 41) of the juveniles born in the field enclosures. All of the juveniles observed that survived until autumn were tested (same individuals in test 2 and test 3). For test 1, we used other individuals that were not observed during their early juvenile phase.

2.3. Experimental housing conditions

For the experiments, we entered the animals singly in small outdoor wire mesh enclosures (360×460 cm^2) with sandy soil. Digging was prevented by a wire mesh layer underneath the sand, and a wire mesh on the top was used to exclude raptors. Each of these

enclosures contained an artificial concrete burrow consisting of a tube (length: 150 cm, diameter: 20 cm) and a chamber with a removable top (diameter: 60 cm). In total, six of these enclosures were available for the experiments.We covered the wire mesh between the different enclosures with wooden blinds in order to prevent social interactions among the individuals. To avoid contact with predators, the whole area was additionally surrounded by a 4 m high wall.

Water was provided *ad libitum* in the center of the enclosure. We placed two wooden boxes (30×30×30 cm^3) within each enclosure as feeding sites for the rabbits. One side of the box was left open, and the rabbits could enter easily. In each box, we placed two concrete feeding bowls. The outer bowl contained food pellets (Solikanin Plus, Ovator, Germany) and in the inner bowl we presented fox feces during the predator odor test (see below). The fox feces was only presented in one of the two boxes, so the animals always had the option to feed at a site without direct contact to fox odor.

The fox feces were collected from captive animals of Hof Zoological Garden (Franconia, Germany). The fresh samples were wrapped inside aluminum foil and were put in plastic bags before being frozen at −20°C. The samples were defrosted shortly before use and soaked in water.

On the top of each of the enclosures, we installed a video camera (monochrome) by which we could observe the whole area. In addition, we fixed a red light bulb (40 W) in each enclosure. This enabled us to videotape the behavior of the animals continuously all-day round during each 24 h period, using time-lapse recorders.

2.4. Experimental procedure

In autumn 2004, juveniles from both field enclosures were captured with peanut-baited traps, which were set in the early morning. We checked the traps from the distance every 30 min to ensure a very low retention time of the trapped animals. The animals were stored singly in gunnysacks in a silent and dark room until six of the focal animals were captured. Then, these animals were entered singly into the concrete warrens of the experimental enclosures. We first blocked the entrances of the warrens with gunnysacks, and waited for 30 min in order to provide the animals enough time to recover from the

transport to this novel environment. Then, we simultaneously opened the entrances of the burrows of all six enclosures, and left the animals undisturbed by any human presence.

2.4.1. Novel environment test

Only 14 out of the 41 focal animals which were observed in the semi-natural enclosure survived until autumn, when we started with the experiments. These animals (8 males, 6 females, Table 1) were video-recorded for the first 24 h after entering into the experimental enclosures. We focused on two variables in order to describe their behavioral response to this novel environment test.

Start of activity: The lapse of time between the opening of the burrow of the experimental enclosure and the moment when the animal left the burrow for the first time.

Investigation: The duration of time outside the burrow that the animal spent moving, or stopping while sniffing and looking around.

2.4.2. Predator odor test

2.4.2.1. Evaluation experiment. For this evaluation experiment, we used an independent sample of 25 juveniles (17 females, 8 males) to evaluate the relationship between the behavioral and physiological responses to the predator odor test (Table 1). In a recent study, it had been shown by us that European rabbits respond to the presence of fox odor by an increase in scanning behavior and also by an increase in their serum corticosterone levels (Monclús *et al.* 2005; Monclús *et al.* 2006). The 25 focal animals for our evaluation test were also trapped in the field enclosures, however, they were not subjected to observational studies during their early juvenile period. We also did not record their behavior during the first day after entering them into the experimental enclosures, although all juveniles were treated in the same way during the whole experiment.

2.4.2.2. Test procedure. In total, the test procedure of the predator odor test was done with 25 animals (evaluation experiment, see above) and the 14 animals which were

previously observed during their early juvenile phase and underwent the novel environment test (Table 1).

This test lasted for 10 days and started 10 days after entering the animals into the experimental enclosures. On the first day of this test, we trapped the animals and measured their physiological stress response by using an ACTH challenge test, as described below. On the 4th day, we video recorded the behavior of the animals for 24 h in order to get basal values of their individual behavioral patterns. We also checked over several days which of the two feeding boxes was preferred by each individual. Preferences were assessed by the amount of food left in the bowls of both feeding boxes; the food pellets were weighed and renewed every morning. In the morning of the 6th day, we then confronted the animals with the odor of a potential predator by presenting fox feces in the empty bowl next to the feeding bowl of the preferred box, and again video recorded the animals for 24 h. We then confronted the animals with the fox odor for another 4 days, whereas we exchanged the position of the two feeding boxes (box with and without fox odor) after 2 days. On the last day, we again trapped the animals and performed a second ACTH challenge test. We determined and calculated two variables:

Change in corticosterone: The percentage change in serum corticosterone challenge levels, measured at the 1st and the 10th day of the predator odor test. Positive changes can be interpreted as an increased secretory capacity of the adrenal glands while negative changes reflect a decrease.

Scanning: The percentage time when the animals showed any signs of alertness while feeding. This included raising the head, turning around, looking at both sides, looking back, or standing at the hind legs. For the calculation, we summed up the total feeding time (=summed up duration of all feeding bouts) per 24 h and measured the duration of all scanning events while the animals were feeding. The duration of one feeding bout was defined as the period of time when a rabbit started to feed until it started another activity or left the proximity of the feeding box. A feeding bout was not terminated by the interruptions of feeding due to scanning events, when the animal did not leave the proximity of the feeding box.

2.5. ACTH challenge test

We injected the animals intramuscularly with 0.1 ml of synthetic ACTH solution (Synacthen Depot, Novartis, Germany; concentration: 1 mg/ml), which results in a maximum increase in glucocorticoid levels (Faulborn *et al*. 1979). The levels (cortisol and corticosterone) reach a maximum plateau 60 min after injection. This maximum level can be interpreted as the adaptive state of an individual's adrenocortical system (i.e. functionality of the adrenal glands), and therefore retrospectively provides information about the animal's physiological stress response during the preceding days (von Holst 1998, pp. 22–29). After injection, the animals were placed separately in gunny sacks (60×90 cm2), and 60 min later blood samples were taken from the marginal ear vein (300 µl) by needle puncture. Bloodwas immediately centrifuged twice and the serum was frozen at −70 °C. Serum corticosterone challenge levels in the samples were analyzed using a radioimmunoassay (Foster & Dunn 1974).

2.6. Statistical analyses

For analysis, we used ANCOVA models. The response variables *start of activity*, *investigation*, and *scanning* were logtransformed prior to the analysis in order to approximate them to a normal distribution (Shapiro–Wilk's test). We used the behavioral variables measured during the juvenile period (covariates: agonistic behavior, positive social behavior; fixed factor: offensive behavior) as predictors and also included sex as a fixed factor. We ensured that variances were homogenous (Levene test). We also calculated all two-way interactions of sex and all behavioral variables considered. If interaction terms were not statistically significant, they were removed and the models were recalculated (Enqvist 2005). In case ANCOVA revealed a significant relationship between the response variable and the covariable, we calculated a Spearman rank correlation between them, using untransformed data. In order to evaluate consistencies in the responses during the two experimental tests (novel environment, predator odor), we tested for bivariate correlations (Spearman rank) between the behavioral variables (start of activity, investigation) measured during the novel environment test and the responses during the predator odor test (scanning behavior, changes in serum corticosterone challenge levels). All analyses were done with SPSS 12.0 (SPSS Inc.).

3. RESULTS

3.1. Social interactions in semi-natural environment

The juveniles (n = 41) were observed in close proximity (< juvenile body length) to other animals of their social group during 13.20% ± 1.13 S.E. of the observation time. This positive ocial behavior was displayed with adults (males and females) during 4.16% ± 0.44 S.E. of time, and also with other juveniles during 9.88% ± 1.08 S.E. of time. The juveniles were on average involved in 0.48 ± 0.09 S.E. agonistic interactions/h. These interactions took place with other juveniles (49.9% of cases) and adults (50.1% of cases). Agonistic interactions with adults were always defensive. Nearly all of the agonistic interactions that juveniles received by adults were initiated after the juvenile approached to the adult. The adults hardly showed any escalating offensive aggression (chasing only in 21.2% of cases) against juveniles, but mainly reacted by a short, non-tactile movement leading to the displacement of the juvenile interaction partner. In encounters with other individuals of their age class, juveniles on average received 0.14 ± 0.04 S.E. agonistic interactions/h. In 27 out of the 41 focal animals (65.8%) we did not observe any offensive behavioral interactions (chasing or displacement) against other juveniles during the observation time. In the remaining 14 animals, we observed an average frequency of 0.28 (±0.07 S.E.) offensive agonistic interactions/h. A similar distribution was apparent in the focal animals which were later available for the experiments: we did not observe any offensive behavior in 7 (50.0%) out of this sub-sample of 14 animals. We did not find significant differences between sexes in the chosen behavioral variables used for the analyses below (positive social behavior: $t_{39} = 0.396$, $p = 0.714$; agonistic behavior: $t_{39} = 0.774$, $p = 0.444$; offensive behavior: $\chi^2_{39} = 0.017, p = 0.896$).

3.2. Evaluation of the predator odor test: relationship between behavioral and physiological stress responses

Relationships between scanning behavior (covariable) and changes in the physiological stress response (dependent variable) were evaluated on an independent data set of 25 individuals. Animals that allocated a higher proportion of their time spent feeding

in scanning showed a significantly lower increase in serum corticosterone challenge levels during the test period ($F_{1,22} = 5.361$, $p = 0.030$). The effects of sex ($F_{1,22} = 0.192$, $p = 0.666$) were not significant. The interaction between the factor sex and the covariable scanning was also not significant ($F_{1,21} = 2.877$, $p = 0.105$) and was skipped from the final model. The relationship between the relative change in plasma corticosterone after ACTH challenge and scanning behaviour (untransformed data) is presented in Fig. 1.

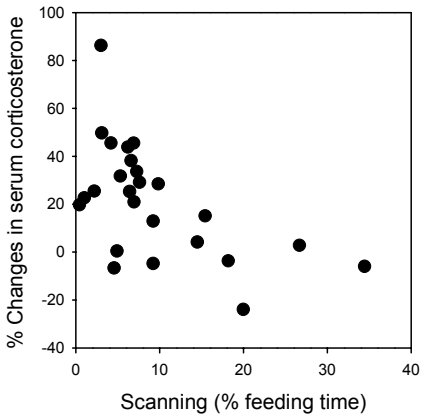

Fig. 1. Correlation between the time spent scanning while feeding when the animals were confronted with fox odor, and the percentage changes in serum corticosterone levels (after ACTH challenge), measured before and after fox odor confrontation (Spearman: $r_s = -0.512$, $n = 25$, $p = 0.009$).

3.3. Social behavior and responses in novel environment test

Individuals, which were involved in more agonistic interactions were quicker to leave their burrow and to start exploration behavior when entered into the experimental enclosures (see Fig. 2).

However, there were no significant relationships between the start of activity and the three behavioral variables recorded during the juvenile phase (Table 2a). The duration of investigation behavior that the animals showed during the first 24 h after being entered into the novel environment was no related to any of the predictors tested (Table 2b). All two-way interactions between sex and the behavioral variables were not significant, and were removed from the final models.

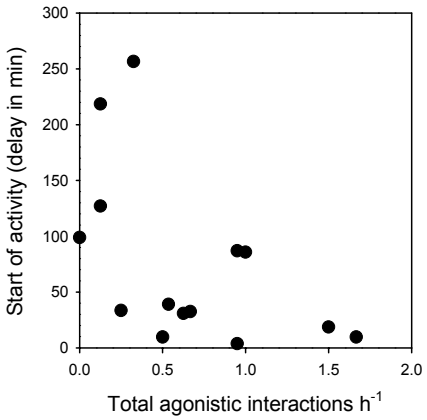

Fig.2. Correlation between the frequency (interactions/h) of agonistic interactions (offensive+defensive) in which the juveniles were involved (with adults+juveniles), measured during their early juvenile period in their social group, and the time delay in the start of activity when they were entered into a novel environment (Spearman: $r_s = -0.637$, $n = 14$, $p = 0.014$).

3.4. Social behavior and responses in predator odor test

Animals which showed a higher degree of positive social behavior by means of a higher proportion of time spent in close proximity to other animals during their early juvenile period, invested more time in scanning when confronted to fox odor (see Fig. 3). The frequency of agonistic interactions, the occurrence of offensive behavior and sex were not related to this behavioral variable (Table 2c). Furthermore, we did not find any effects of the tested predictor variables on the increase in serum corticosterone values (Table 2d). All two-way interactions between sex and the behavioral variables were not significant, and were removed from the final models.

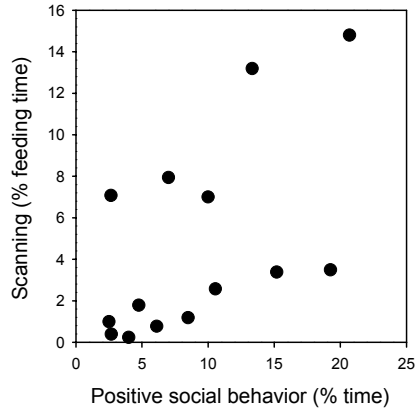

Fig. 3. Correlation between positive social behavior of juveniles (% observation time in close proximity to adults+juveniles), measured during the early juvenile period in their social group, and the time spent scanning while feeding when the animals were kept singly and were confronted with fox odor (Spearman: $r_s = 0.582$, $n = 14$, $p = 0.029$.

3.5. Consistency across both test situations

We did not detect any relationships between both behavioural variables measured during the novel environment test and scanning behavior (dependent variable) when confronted with fox odor (effects of *start of activity*: $F_{1,11} = 0.007$, $p = 0.936$; effects of *investigation*: $F_{1,11} = 0.005$, $p = 0.945$). Also, the behavioral responses during the first test were not related to the physiological stress response (dependent variable) that the animals showed during the second test (effects of *start of activity*: $F_{1,11} = 0.554$, $p = 0.472$; effects of *investigation*: $F_{1,11} = 1.853$, $p = 0.201$).

Test	Response variable	Source of variation	*F*	*p*
Novel environment test	(a) *Start of activity*	Positive social behavior	0.094	0.767
		Agonistic behavior	5.341	0.046 *
		Offensive behavior	0.538	0.482
		Sex	0.013	0.911
	(b) *Investigation*	Positive social behavior	0.077	0.788
		Agonistic behavior	0.010	0.921
		Offensive behavior	0.088	0.773
		Sex	0.114	0.744
Predator odor test	(c) *Scanning*	Positive social behavior	5.239	0.048 *
		Agonistic behavior	0.002	0.970
		Offensive behavior	0.756	0.407
		Sex	0.230	0.643
	(d) *Change in corticosterone*	Positive social behavior	2.867	0.125
		Agonistic behavior	0.549	0.478
		Offensive behavior	2.313	0.163
		Sex	1.124	0.317

Table 2. ANCOVA models of the effects of different behavioral variables (covariates: agonistic behavior, positive social behavior; fixed factors: occurrence of offensive behavior, sex) describing the social interactions of juvenile European rabbits with other juveniles and adults in a natural environment on response variables measured when the juveniles (a, b) were introduced singly into a novel environment and (c, d) were confronted with fox odor (n=14). Significant results are marked with asterisks.

4. DISCUSSION

We investigated the relationships between social interactions during the juvenile phase and the individual behavioral as well as physiological responses to experimental stressors to which the animals were exposed at an age of 3 to 4 months. Overall, we found correlations

proving the existence of such a relationship. Nevertheless, the behavioral responses to experimental stressors were not consistent across both experimental test situations.

We also evaluated the relationship between behavioral and physiological responses of rabbits to the presentation of fox odor by using an independent sample of animals. Our expectation was met; rabbits that scanned more showed a lower increase or even a slight decrease in their serum corticosterone levels. Severely increased corticosterone concentrations of up to 86% over the basal levels only occurred in animals that scanned less. Such strong adrenocortical stress responses are commonly found in passive individuals, which lack control over their current, stressful situation (Henry & Stephens 1977; von Holst 1998). For example, tree shrews that lost a confrontation with a resident animal but kept on displaying active avoidance showed a significantly lower increase in glucocorticoids than passive (submissive) animals (von Holst 1986). We suggest that a high scanning rate under the conditions of our predator odor test could be considered as an important element of an active coping style.

Individuals which were involved at higher frequency in agonistic interactions (defensive+offensive) with adults and other juveniles during their early juvenile period started to explore sooner from their burrows when entered into the novel environment of our test design. The individual levels of aggressiveness, which we categorized by the occurrence of offensive behavior during the juvenile phase, predicted neither how fast nor how long the animals explored the experimental enclosures. At first sight, these results seem to contradict studies on other animals, where more aggressive individuals were commonly reported to adopt a more active behavioral strategy under stressful conditions (Benus *et al.* 1991; Verbeek *et al.* 1996; Koolhaas *et al.* 1999; Bolhuis *et al.* 2005; Carere *et al.* 2005). However, offensive aggression that the juveniles displayed against others during our observation period was rare. Most of the offensive interactions with other juveniles were observed in the beginning of the observations, when the focal animals were only 6–8 weeks old. These interactions might be therefore considered as elements of social play. Our results rather showed that individuals which were bolder in their novelty response were previously involved in more agonistic, mainly defensive interactions against adults. The frequency of the agonistic interactions during the juvenile phase might also be used as a measure of

boldness, since the occurrence of defensive behavior in juveniles was mainly the result of a preceding active approach into the close proximity of adults or older juveniles.

Juveniles were frequently found in close proximity to group members, however the values differed considerably among individuals (range: 2.5% to 21.7% of observation time). Generally, such positive social interactions with conspecifics play an important role during the early social development, since juveniles that manage to integrate successfully into a social group have been shown to possess a better physiological condition and immune status during the winter season (von Holst 1998; Rödel 2000). The results of our second reaction test revealed that rabbits, which were involved in a higher level of positive social interactions during their juvenile phase, responded to the presence of predator odor by comparatively higher scanning rates. We suggest two non-exclusive explanations for this observed relationship. On the one hand, a high rate of positive social behavior with conspecifics might reflect a more active behavioral style of a juvenile, since resting in close proximity, at least to adults, was mainly initiated by the juvenile interaction partner. On the other hand, the higher level of positive social interactions might directly influence how an animal copes with challenging situations during its later life. As yet, not much is known about the determinants of coping styles. Several studies point out that such differences between individuals might be genetically predisposed (Sluyter *et al.* 1996; Carere *et al.* 2003; Ogawa 2004). However, there is growing evidence that the way how an animal behaves or copes with challenge is influenced by experience during its early development (Anisman *et al.* 1998; Sachser 1998; Meerlo *et al.* 1999; Kaiser *et al.* 2003). For example, it has been concluded in a study on the responses of male laboratory rats (*Rattus norvegicus*) to restraint stress or to cat odor, that stressful social experience may cause a shift in their behavior towards more passive forms of defense (Blanchard *et al.* 2001). Our approach, which is based on correlations between the social interactions of juveniles in their natural environment and responses to experimental stressors does not allow to draw any clear conclusions on causal relationships, since the effects of genetic factors and of the early environment cannot be assessed. Despite this, we speculate that social support by group members during the early development, which might have improved the animal's capability to act in a more (pro)active way, could be an important mechanism underlying the correlations observed.

Based on our results on the evaluation of the predator odor test, we expected that the higher vigilance in animals that were involved in more positive social interaction during their juvenile phase should also entail a lower increase in their adrenocortical stress response. However, we did not find such a negative correlation. A possible reason might be the differences in the distribution of the two data sets. Individuals with extremely high scanning rates, as we could observe in the sample that we used for the test evaluation (see Fig. 1) did not occur in the sample of our experimental animals (see Fig. 3).

Under natural conditions, and also under the semi-natural conditions of our field enclosures, the mortality rate of juvenile rabbits is around 30–80% during the following weeks after first emergence above ground, mainly due to predation and diseases (Tyndale-Biscoe 1955; Parer 1977; Richardson & Wood 1982; Cowan 1987**)**. For this reason, it was difficult for us to get a sufficiently high number of study animals for our reaction tests, and therefore we relied on animals of both sexes. However, all animals were tested several months before they usually take part in the reproductive process. Our analyses revealed that sex did not play any relevant role for the behavioral patterns studied. Neither social behavior during the juvenile period nor any of the responses in our two tests differed statistically between males and females. Similar results have been found in other studies (Marchetti & Drent 2000; Dingemanse *et al.* 2002).

The results of our study failed to support individual consistencies in the behavioral responses across both experimental test situations. Faster explorers in the novel environment test did not show a more active behavioral style during the predator odor test by means of higher scanning. In addition, the patterns of social behavior which were correlated with the reactivity in one of the test situations did not explain variation in the other and vice versa. Such inconsistencies have also been repeatedly observed in other studies. For example, no correlations were found between behavioral responses of domestic pigs to challenging situations in social and non-social contexts (Forkmann *et al.* 1995; Spooler *et al.* 1996; D'Eath & Burn 2002; but see Bolhuis *et al.* 2005). This leads to the question whether the existence of specific behavioral styles can be generalized across situations, or might be rather context specific (Wilson *et al.* 1994**)**. It has been suggested that more than one underlying behavioral dimension might be involved in controlling the responses to social and non-social challenges in domestic pigs (Lawrence *et al.* 1991). This might also apply to

European rabbits, at least with respect to behavioural responses in the different test situations used in our study. We speculate that a fast exploration from the shelter of the artificial burrow into the novel environment of the experimental enclosure might primarily show the boldness or curiosity of an animal, whilst a high scanning rate in the presence of the odor of a potential predator may mainly reflect a more (pro) active coping style. Further evidence comes from other studies in animals including humans which emphasize the multidimensional character of individual personality traits (Ursin 1980; Steimer *et al.* 1991; Gosling 2001). For example, it has been suggested that the personality of spotted hyenas can be described with high reliability by five different dimensions (Gosling 1998). However, it could also be argued that the response patterns of the rabbits might be "domain specific", meaning that the rabbits have displayed differential behavioural styles during the different experimental situations, or more generally, that an animal may express one or another behavioural disposition (e.g. shyness or boldness) depending on the environmental domain or context (Wilson 1998).

In conclusion, the findings of our study did not facilitate a clear and consistent discrimination of behavioral phenotypes in European rabbits during different test situations. Faster explorers in the novel environment test did not show a more active behavioral style during the predator odor test. However, we could show that behavioral traits during the early social development were correlated with specific behavioral responses during later life.

Acknowledgements

We thank Tanja Scherbel, Barbara Bruchner, Christina Schott, Andrea Schlenk, Manuela Zapka, Anett Starkloff and Rowena Wychoff who helped in the data collection, and Karl-Heinz Pöhner and Kurt Bayer for animal care. Thanks to Inge Zerenner-Fritzsche for the hormone analyses, to the staff of the Zoological Garden in Hof for the collection of the fox feces, and to Dr. Wolf Stöhr and Dr. Frank Uhl who kindly provided technical support during the video recording sessions. We are grateful to three anonymous reviewers for their

very helpful comments on our manuscript. Permission for population biology studies on European rabbits and for the ACTH challenge test was granted by the government of Middle Franconia (211-3894a; 621-2531.32-5/05). The project was supported by a grant from the Deutsche Forschungsgemeinschaft (Ro2431/2-1).

REFERENCES

Anisman, H., Zaharia, M. D., Meaney, M. J., & Merali, Z. 1998. Do early life events permanently alter behavioral and hormonal responses to stressors? *International Journal of Developmental Neuroscience*, **16**: 149–164.

Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A. & McGregor, I. S. 2005 The effects of predator odors in mammalian prey species: a review of field and laboratory experiments. *Neuroscience and Biobehavioral Reviews*, **29**: 1123–1144.

Armitage, K. B. 1986. Individuality, social behavior, and reproductive success in yellow-bellied marmots. *Ecology*, **67**: 1186–1193.

Armitage, K. B. & Van Vuren, D. H. 2003. Individual differences and reproductive success in yellow-bellied marmots. *Ethology Ecology & Evolution*, **15**: 207–233.

Barrett, L. & Henzi, S. P. 2002. Constraints on relationship formation among female primates. *Behaviour*, **139**: 263–289.

Benus, R. F., Koolhaas, J. M. & van Oortmerssen, G. A. 1987. Individual differences in behavioural reaction to a changing environment in mice and rats. *Behaviour*, **100**: 105–122.

Benus, R. F., Bohus, B., Koolhaas, J. M. & Van Oortmerssen, G. A. 1991. Heritable variation for aggression as a reflection of individual coping strategies. *Experientia*, **47**: 1008–1019.

Blanchard, R. J., Yudko, E., Dulloog, L. & Blanchard, D. C. 2001. Defense changes in stress nonresponsive subordinate males in a visible burrow system. *Physiology and Behavior*, **72**: 635–642.

Bolhuis, J. E., Schouten, W. E. P., Schrama, J. W. & Wiegants, V. M. 2005. Individual coping characteristics aggressiveness and fighting strategies in pigs. *Animal Behaviour*, **69**: 1085–1091.

Campbell, T., Lin, S., DeVries, C. & Lambert, K. 2003. Coping strategies in male and female rats exposed to multiple stressors. *Physiology and Behavior*, **78**: 495–504.

Carere, C., Groothuis, T. G. G., Möstl, E., Daan, S. & Koolhaas, J. M. 2003. Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and the response to social stress. *Hormones & Behaviour*, **43**: 540–548.

Carere, C., Drent, P. J., Privitera, L., Koolhaas, J. M. & Groothius, T. G. G. 2005. Personalities in great tits *Parus major* stability and consistency. *Animal Behaviour*, **70**: 790–805.

Cavigelli, S. A. & McClintock, M. K. 2003. Fear of novelty in infant rats. *Proceedings of the National Academy of Science*, *USA*, **100**: 16131–16136.

Clarke, A. S. & Boinski, S. 1995. Temperament in nonhuman primates. *American Journal of Primatology*, **37**: 103–125.

Corson, S. A. & Corson, E. O. 1976. Constitutional differences in physiologic adaptation to stress and distress. In: Serbum G, (Ed.). *Psychopathology of human adaptation*, pp. 77–94. New York: Plenum Press.

Cowan, D. P. 1987. Aspects of the social organisation of the European rabbit (*Oryctolagus cuniculus*). *Ethology*, **75**: 197–210.

Cowan, D. P. 1987. Patterns of mortality in a free-living rabbit (*Oryctolagus cuniculus*) population. *Symposium of the Zoological Society London*, **58**: 59–70.

D'Eath, R. B. & Burn, C. 2002. Individual differences in behaviour: a test of 'coping styles' does not predict resident–intruder aggressiveness in pigs. *Behaviour*, **139**: 1175–1194.

Dingemanse, N. J., Both, C., Drent, P. J., Van Oers, K. & Van Noordwijk, A. J. 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour*, **64**: 929–938.

Engqvist, L. 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour*, **70**: 967–971.

Faulborn, K. W., Fenske, M., Pitzel, L. & König, A. 1979. Effects of an intravenous injection of tetracosactid on plasma corticosteroid and testosterone levels in unstressed male rabbits. *Acta Endocrinologica*, **91**: 511–518.

Forkmann, B., Furuhang, I. L. & Jensen, P. 1995. Personality coping patterns and aggression in piglets. *Applied Animal Behaviour Science*, **45**: 31–42.

Foster, L. B. & Dunn, R. T. 1974. Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clinical Chemistry*, **20**: 365–368.

Gosling, S. D. 1998. Personality dimensions in spotted hyenas (*Crocuta crocuta*). *Journal of Comparative Psychology*, **112**: 107–118.

Gosling, S. D. 2001 From mice to men: what can we learn about personality from animal research? *Psychological Bulletin*, **127**: 45–86.

Groothuis, T. G. G. & Carere, C. 2005. Avian personalities: characterization and epigenesis. *Neuroscience and Biobehavioral Reviews*,**29**: 137–150.

Gust, D. A., Gordon, T. P.,Wilson, M. E., Brodie, A. R., Ahmed-Ansari, A. & McClure, H. M. 1996. Group formation of female pigtail macaques (*Macaca nemestrina*). *American Journal of Primatology*, **39**: 263–273.

Hennessy, M. B. 1997. Hypothalamic–pituitary–adrenal responses to brief social separation. *Neuroscience and Biobehavioral Reviews*, **21**: 11–29.

Henry, J. M. & Stephens. & P. M. 1977. *Stress health and the social environment: a sociobiological approach to medicine*. Berlin: Springer.

Hessing, M. J. C., Hagelsø, A. M., van Beek, J. A. M., Wiepkema, P. R. & Schouten, W. P. G. 1994. Individual behavioral and physiological strategies in pigs. *Physiology & Behavior*, **55**: 39–46.

Huntingford, F. A. 1976. The relationship between antipredator behaviour and aggression among conspecifics in the three spine stickleback, *Gasterosteus aculeatus*. *Animal Behaviour*, **24**: 245–260.

Kagan, J., Reznick, J. S. & Snidman, N. 1988. Biological basis of childhood shyness. *Science*, **240**: 167–171.

Kaiser, S., Kruijver, F. P. M., Straub, R. H., Sachser, N. & Swaab, D. F. 2003. Early social stress in male guinea-pigs changes social behaviour, and autonomic and neuroendocrine functions. *Journal of Neuroendocrinology*, **15**: 761–769.

Kats, L. B. & Dill, L. M. 1998. The scent of death: chemosensory assessment of predation risk by prey animal. *Ecoscience*, **5**: 361–394.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van der Vegt, B. J., Van Reenen, C. G., Hopster, H., De Jong, I. C., Ruis, M. A. W. & Blokhuis, H. J. 1999. Coping styles in animals current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews*, **23**: 925–935.

Koolhaas, J. M., De Boer, S. F., Buwalda, B., Van der Vegt, B. J., Carere, C. & Groothius, A. G. G. 2001. How and why coping systems vary among individuals. In: Broom, D. M. (Ed.). *Coping with challenge. Welfare in animals including humans*, p. 197–210. Berlin: Dahlem Workshop Report 87 Dahlem University Press.

Lawrence, A. B., Terlouw, E. M. C. & Illius, A. W. 1991. Individual differences in behavioural responses of pigs exposed to non-social and social challenges. *Applied Animal Behaviour Science*, **30**: 73–86.

Lima, S. L. 1998. Stress and decision making under the risk of predation: recent developments from behavioral reproductive and ecological perspectives. *Advances in the Study of Behavior*, **27**: 215–290.

Lockley, R. M. 1961. Social structure and stress in the rabbit warren. *Journal of Animal Ecology*, **30**: 385–423.

Macrí, S., Mason, G. J. & Würbel, H. 2004. Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. *European Journal of Neuroscience*, **20**: 1017–1024.

Marchetti, C. & Drent, P. J. 2000. Individual differences in the use of social information in foraging by captive great tits. *Animal Behaviour*, **60**: 131–140.

Martin, P. & Bateson, P. 1986. *Measuring behaviour*. Cambridge: Cambridge University Press; 1986.

Meerlo, P., Horvath, K. M., Nagy, G M., Bohus, B. & Koolhaas, J. M. 1999. The influence of postnatal handling on adult neuroendocrine and behavioural stress reactivity. *Journal of Neuroendocrinology*, **11**: 925–933.

Monclús, R., Rödel, H. G., von Holst, D. & de Miguel, J. 2005. Behavioural and physiological responses of naïve European rabbits to predator odour. *Animal Behaviour*, **70**: 753–761.

Monclús, R., Rödel, H. G., Palme, R., von Holst, D. & de Miguel, J. 2006. Non-invasive measurement of the physiological stress response of wild rabbits to the odour of a predator. *Chemoecology*, **16**: 25–29.

Ogawa, S., Choleris, E. & Pfaff, D. 2004. Genetic influences on aggressive behaviours and arousability in animals. *Annals of the New York Academy of Science* **1036**: 257–266.

Parer, I. 1977. The population ecology of the wild rabbit, *Oryctolagus cuniculus* (L.), in a Mediterranean type climate in New SouthWales. *Australian Wildlife Research*, **4**: 171–205.

Pervin, L. & John, O. P. 1997. *Personality: theory and research*. NewYork:Wiley.

Réale, D., Gallant, B. Y., Leblanc, M. & Festa-Bianchet, M. 2000. Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Animal Behaviour*, **60**: 589–597.

Richardson, B. & Wood, D. H. 1982. Experimental ecological studies on a subalpine rabbit population: I. Mortality factors acting on emergent kittens. *Australian Wildlife Research*, **9**: 443–450.

Rödel, H. G. 2000. Low temperature effects and social influences on physiological condition of subadult wild rabbits. In: Heldmaier, G. & Klingenspor, M. (Eds). *Life in the cold*, pp. 511–518. Berlin: Springer Verlag.

Sachser, N. 1998. Of domestic and wild guinea pigs: studies in sociophysiology domestication and social evolution. *Naturwissenschaften*, **85**: 307–317.

Sih, A., Bell, A. & Johnson, J. C. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution*, **19**: 372–378.

Sih, A., Bell, A., Johnson, J. C. & Ziemba, R. E. 2004. Behavioral syndromes: an integrative overview. *Quartertly Review of Biology*, **79**: 241–279.

Sluyter, F., van der Vlugh, J. J., Van Oortmerssen, G. A., Koolhaas, J. M., van der Hoeven, F. & De Boer, P. 1996. Studies on wild house mice: VII. Prenatal maternal environment and aggression. *Behavior Genetics*, **26**: 512–518.

Spooler, H. A. M., Burbidge, J. A., Lawrence, A B., Simmins, H. & Edwards, S. A. 1996. Individual behavioural differences in pigs: intra- and inter-test consistency. *Applied Animal Behaviour Science*, **49**: 185–198.

Steimer, T., La Fleur, S. & Schulz, P. E. 1991. Neuroendocrine correlates of emotional reactivity and coping in male rats from the Roman high (RHA/Verh)- and low (RLA/Verh)-avoidance lines. *Behavior Genetics*, **27**: 503–512.

Suomi, S. J. 1991. Uptight and laid-back monkeys: individual differences in the response to social challenges. *In*: Brauth, S. E., Hall, W. S. & Dooling, R. J., (Eds.). *Plasticity of development*, pp. 27–56. Cambridge, Massachusetts: MIT Press.

Takahashi, L. K., Nakashima, B. R., Hong, J. & Watanabe, K. 2005. The smell of danger: a behavioral and neural analysis of predator odor-induced fear. *Neuroscience and Biobehavioral Reviews*, **29**: 1157–1167.

Tyndale-Biscoe, C. H. & Williams, R. M. 1955. A study of natural mortality in a wild population of the rabbit. *New Zealand Journal of Science and Technology*, **36**: 561–580.

Ursin, H. 1980. Personality activation and somatic health: a new psychosomatic theory. *In*: Levine, S., Ursin, H. (Eds.). *Coping and health*, pp. 259–279. New York: Plenum Press.

Veenema, A. H., Meijer, O. C., de Kloet, E. R., Koolhaas, J. M. & Bohus, B. G. 2003. Differences in basal and stress-induced HPA regulation of wild house mice selected for high and low aggression. *Hormones and Behavior*, **43**: 197–204.

Verbeek, M. E. M., Drent, P. J. & Wiepkema, P. R. 1994. Consistent individual differences in early exploratory behaviour ofmale great tits. *Animal Behaviour*, **48**: 1113–1121.

Verbeek, M. E. M., Boon, A. & Drent, P. J. 1996. Exploration aggressive behaviour and dominance in pair-wise confrontations of juvenile male great tits. *Behaviour*, **133**: 945–963.

von Holst, D. 1986. Psychosocial stress and its pathophysiological effects in tree shrews (*Tupaia belangeri*). *In*: Schmidt, T. H., Dembroski, T. M. & Blümchen, G., (Eds.). *Biological and psychological factors in cardiovascular disease*, pp. 477–489 Berlin: Springer Verlag.

von Holst, D. 1998. The concept of stress and its relevance for animal behavior. *Advances in the Study of Behavior*, **27**: 1–131.

Wilson, D. S., Clark, A. B., Coleman, K. & Dearstyne, T. 1994. Shyness and boldness in humans and other animals. *Trends in Ecology and Evolution*, **9**: 151–162.

Wilson, D. S. 1998. Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society, London B*, **353**: 199–205.

CAPÍTULO 7

Discusión general y perspectivas

Los resultados obtenidos indican que los conejos reconocen a los depredadores por el olor. Para que se produzca el reconocimiento oloroso de los depredadores hace falta una larga historia evolutiva entre la presa y sus depredadores (Stoddart, 1980; Apfelbach *et al*. 2005). Es el caso del conejo, que durante toda su historia evolutiva ha estado sometido a una presión de depredación alta. En la actualidad, se estima que en ambientes mediterráneos unas 40 especies depredan sobre él (Delibes e Hiraldo 1981; Jaksic y Soriguer 1981). Por lo tanto, no es de extrañar que el reconocimiento de los depredadores por el olor sea independiente de la experiencia en el conejo, ya que el reconocimiento mediante aprendizaje sería demasiado peligroso. Sin embargo, Ferrari y colaboradores (2007), sugieren que en aquellos casos en los que las presas están sometidas a unos pocos depredadores y la capacidad de predecir un ataque alta, las presas se beneficiarían de mecanismos innatos de reconocimiento de depredadores. Por el contrario, en los casos en los que la diversidad de depredadores es alta y la capacidad de predecir un ataque baja, sería beneficioso para la presa el reconocimiento aprendido y generalizado de los depredadores. Creemos que en el caso del conejo, dada la gran variedad de depredadores que tienen y su diversidad taxonómica, los posibles costes del aprendizaje del reconocimiento de sus depredadores serían mayores que los derivados de las respuestas a estímulos que no supongan un riesgo real. Además, como hemos comprobado en este estudio, los juveniles, a los que se les supondría una respuesta menos específica (Inglis 1979; Vitale 1989), mostraron un comportamiento similar al de los adultos, lo que sugiere que el mecanismo se refina muy pronto en la vida de los conejos (Pongrácz y Altbäcker 2000).

En los conejos el comportamiento antidepredatorio consistió en incrementar la vigilancia (detección del depredador). Éste es un comportamiento general, que cabría esperar en aquellos animales cuyo rango de depredadores potenciales es grande, mientras que si estuvieran sometidos a un rango estrecho de depredadores, el comportamiento desarrollado sería específico para esos depredadores (Grostal y Dicke 2000). Mediante la vigilancia, los animales adquieren información sobre su entorno. Sería decisiva en los primeros estadíos y su principal función sería evitar los posibles encuentros directos con los depredadores y controlar la presencia del depredador una vez que se hubiera producido el encuentro (Lima y Dill 1990; Kavaliers y Choleris 2001). Debido a que interviene en las primeras etapas de la secuencia de la depredación, en las que la probabilidad de que una presa escape con éxito es mayor, el éxito o el fracaso de

un animal a la hora de evitar a los depredadores, podría estar íntimamente ligada al desarrollo de esta actividad. Además existen evidencias de que la vigilancia podría constituir una señal disuasoria para los depredadores potenciales. Los depredadores que detecten la señal evitarán atacar a las presas vigilantes (Scannell *et al*. 2001).

La vigilancia, aparte de ser un comportamiento antidepredatorio, desempeña funciones sociales. Los conejos tienen que controlar su entorno para detectar la presencia de posibles competidores. Los depredadores y los competidores difieren en el riesgo potencial que suponen y en la distancia a la que se encuentran, por lo que la vigilancia que exhiben para detectarlos y controlar sus movimientos también es diferente. Por una parte, desarrollan una vigilancia de alta intensidad hacia los depredadores, que consiste en exploraciones largas y poco frecuentes. Para ello adquieren una postura erguida, que probablemente sirva como señal disuasoria de la depredación. Y por otra parte, desarrollan una vigilancia de baja intensidad hacia los congéneres que se encuentran en el grupo, y mediante exploraciones cortas y frecuentes controlan la posición y los movimientos de éstos, tratando de evitar o propiciar agresiones. Estos dos comportamientos podrían conllevar costes diferentes, dado que cuando los conejos exhiben la vigilancia de alta intensidad dejan de comer. Sin embargo, creemos que en un herbívoro como el conejo, en el que la distribución del recurso alimenticio es continua, la pérdida de algunos minutos en la alimentación puede ser fácilmente compensada. En condiciones de laboratorio, los conejos retrasaron el comienzo de la alimentación, pero lo compensaron a lo largo del día, dando como resultado una adquisición similar de energía en presencia y en ausencia de depredador.

La respuesta antidepredatoria exhibida en situaciones de alto riesgo de depredación fue independiente del sexo y de la edad de los individuos. Sin embargo, la tasa basal de vigilancia de los juveniles fue menor, lo que podría explicar su mayor mortalidad ante depredadores aéreos (Rödel *et al*. 2007). Los machos y las hembras no mostraron diferencias en cuanto a las respuestas comportamentales y fisiológicas exhibidas. Esto contrasta con otros estudios en los que se ha encontrado una tasa de vigilancia diferente para cada uno de los sexos, especialmente en la época reproductiva (Renouf & Lawson 1986; Loughry 1993; Cameron y du Toit 2005). Además, las diferencias sexuales en los niveles de glucocorticoides se han destacado en muchas especies (revisado en Touma y Palme 2005). Sin embargo, en nuestro estudio, los conejos se enfrentaban a un riesgo alto de depredación, y aunque los niveles basales diferían, la respuesta tanto comportamental como fisiológica fue similar.

El comportamiento antidepredatorio exhibido por los conejos en condiciones de laboratorio y en condiciones seminaturales fue similar. Sin embargo, nuestros resultados son contradictorios en cuanto a la respuesta fisiológica exhibida. En condiciones de laboratorio los conejos que encontraron un depredador simulado incrementaron los niveles de corticosterona en sangre y de metabolitos de la corticosterona en heces. Esta respuesta, sin embargo, no se encontró en condiciones seminaturales. Se han encontrado resultados similares en otros trabajos (Cockrem y Silverin 2002; Ylönen *et al*. 2006). Una posible causa sería metodológica. En el experimento en condiciones seminaturales, la respuesta fisiológica se midió exclusivamente en los subadultos, debido a que no conseguimos recapturas suficientes de adultos. Otra explicación posible sería que los animales en condiciones naturales poseen muchos mecanismos para controlar su situación (Cockrem y Silverin 2002; DeVries *et al*. 2003), lo que podría tamponar la respuesta de estrés. Sin embargo, la mayoría de los trabajos en los que no se ha detectado una respuesta fisiológica como consecuencia del encuentro con un depredador, han utilizado la presencia simulada de los depredadores, bien por el olor o por presentar a los animales modelos de depredadores (disecados). Por lo tanto, la falta de un refuerzo, es decir, la falta de un encuentro directo con el depredador, podría ser la responsable de la ausencia de la respuesta fisiológica de estrés.

Sería interesante analizar las causas de esta falta de consistencia en la respuesta fisiológica de estrés. Probablemente, la respuesta la encontraríamos en condiciones naturales, donde los conejos están sometidos a un riesgo real de depredación. En este caso, el uso de la técnica no invasiva que hemos validado constituiría una herramienta muy útil para la caracterización de la respuesta fisiológica a los depredadores, ya que la captura y recaptura de los animales no suele ser fácil. Además, se ha comprobado en numerosas especies la validez de estos métodos (Lane 2006).

Con el trabajo realizado hemos contestado a una serie de preguntas sobre el efecto de los depredadores en el comportamiento y la fisiología del conejo. Sin embargo, quedan muchos temas pendientes y mucho trabajo por hacer. Por un lado, sería interesante saber si existe una relación entre el comportamiento antidepredatorio y el efecto directo de los depredadores (consumo) y cómo afecta este último a la estructura de las poblaciones. Por otro lado, sería importante conocer las consecuencias a medio y a largo plazo del comportamiento antidepredatorio en el éxito biológico de las presas (los efectos indirectos de los depredadores). Sólo conociendo los efectos directos e indirectos

de los depredadores podríamos conseguir una imagen aproximada de la importancia de la depredación en las especies presa.

REFERENCIAS BIBLIOGRÁFICAS

Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A. y McGregor, I. S. 2005. The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neuroscience and Biobehavioral Reviews*, **29**: 1123–1144.

Cameron, E. Z. y du Toit, J. T. 2005. Social influences on vigilance behaviour in giraffes, *Giraffa camelopardalis*. *Animal Behaviour*, **69**: 1337–1344.

Cockrem, J. F. y Silverin, B. 2002. Sight of a predator can stimulate a corticosterone response in the great tit (*Parus major*). *General and Comparative Endocrinology*, **125**, 248—255.

Delibes, M., e Hiraldo, F. 1981. The rabbit as prey in the Iberian mediterranean ecosystem. *En* Myers, K. y MacInnes, C. D. (Eds.). *Proceedings of the World Lagomorph Conference*, pp. 614–622. Ontario: University of Guelph.

DeVries, A. C., Glasper, E. R. y Detillion, C. E. 2003: Social modulation of stress responses. *Physiology and Behavior*, **79**: 399–407.

Ferrari, M. C. O., Gonzalo, A., Messier, F. y Chivers, D. P. 2007. Generalization of learned predator recognition: an experimental test and framework for future studies. *Proceeding of the Royal Society B*, **274**: 1853–1859.

Grostal, P. y Dicke, M. 2000. Recognising one's enemies: a functional approach to risk assessment by prey. *Behavioral Ecology and Sociobiology*, **47**: 258–264.

Inglis, I. R. 1979: Visual bird scarers an ethological approach. *En*: Wright, E. N., Inglis, I. R. y Feare, C. J. (Eds.). *Bird Problems in Agriculture*, pp. 121—143. London: MAFF Publishers.

Jaksic, F. M. y Soriguer, R. C. 1981. Predation upon the European rabbit (*Oryctolagus cuniculus*) in Mediterranean habitats of Chile and Spain: a comparative analysis. *Journal of Animal Ecology*, **50**: 269–281.

Kavaliers, M. y Choleris, E. 2001. Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neuroscience and Biobehavioral Reviews*, **25**: 577–586.

Lane, J. 2006. Can non–invasive glucocorticoid measures be used as reliable indicators of stress in animals? *Animal Welfare*, **15**: 331–342.

Lima, S. L. y Dill, L. M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**: 619–640.

Loughry, W. J. 1993. Determinants of time allocation by adult and yearling black–tailed prairie dogs. *Behaviour*, **124**: 23–43.

Pongrácz, P. y Altbäcker, V. 2000. Ontogeny of the responses of European rabbits (*Oryctolagus cuniculus*) to aerial and ground predators. *Canadian Journal of Zoology*, **78**: 655–665.

Renouf, D. y Lawson, J. W. 1986. Harbour seal vigilance: Watching for predators or mates? *Biology of Behaviour*, **11**: 44–49.

Rödel, H. G., Starkloff, A., Bautista, A., Friedrich, A. C. y von Holst, D. 2007. Infanticide and maternal offspring defence in European rabbits under natural breeding conditions. *Ethology*, (in press).

Scannell, J., Roberts, G. y Lazarus, J. 2001. Prey scan at random to evade observant predators. *Proceedings of the Royal Society of London. Serie B*, **268**: 541–547.

Stoddart, D. M. 1980. *The ecology of vertebrate olfactation*. London: Chapman and Hall.

Vitale, A. F. 1989. Changes in anti–predator responses of wild rabbits, *Oryctolagus cuniculus* (L.), with age and experience. *Behaviour*, **110**: 47–61.

Ylönen. H., Eccard, J. A., Jokinen. I. y Sundell, J. 2006. Is the antipredatory response in behaviour reflected in stress measured in faecal corticosteroids in a small rodent? *Behavioral Ecology and Sociobiology*, **60**: 350–358.

CONCLUSIONES GENERALES

Los conejos son capaces de reconocer a los depredadores por el olor, aun sin encuentros previos con los mismos.

La respuesta desencadenada por el reconocimiento consiste en modificaciones comportamentales que actúan en los primeros estadíos de la secuencia de la depredación.

Las respuestas a los depredadores son independientes del sexo y de la edad de los conejos. Sin embargo, los acontecimientos que suceden durante las primeras etapas de la vida del conejo, pueden modular el comportamiento que exhibirán posteriormente en su vida.

Dado que la presencia tanto real como simulada de los depredadores no produjo modificaciones en las actividades de mantenimiento o en la alimentación de los conejos, su comportamiento antidepredatorio podría representar una estrategia de bajo coste, por lo que podría conservarse aun en ausencia de depredadores.

El principal comportamiento antidepredatorio de los conejos, la vigilancia, juega un papel importante en la dinámica del grupo. La vigilancia frente a depredadores y competidores, que representan desiguales niveles de riesgo, difiere cuantitativa y cualitativamente, por lo que podrían considerarse dos comportamientos diferentes.

Aparentemente, en condiciones naturales el umbral de respuesta fisiológica de alarma es más alto, aunque se necesitan nuevos experimentos para confirmarlo. Creemos que la técnica no invasiva validada en este trabajo será fundamental para contestar a esta pregunta pendiente.